Determination of minimal media requirements for *Campylobacter jejuni*

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Norwich Research Park
Norwich, UK

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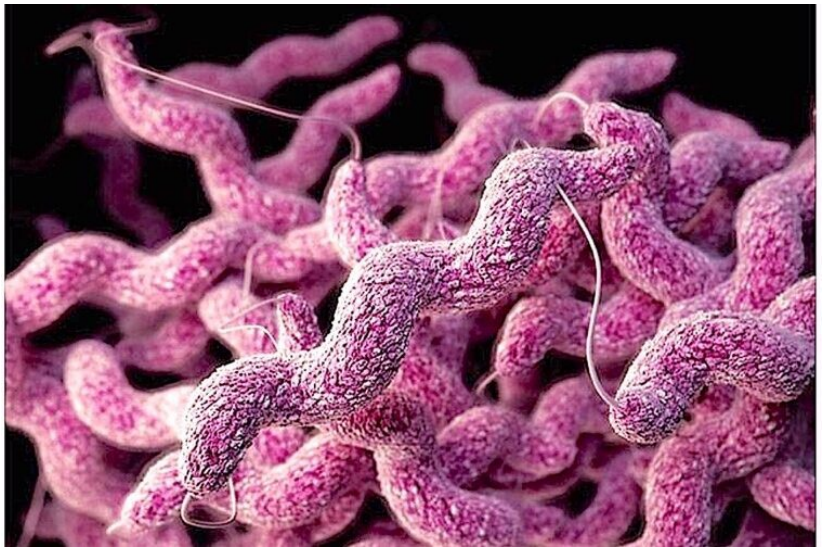
Campylobacter

- Gram negative, microaerophilic, curved or spiral shaped bacteria



- **Main causes of human gastroenteritis worldwide:**
  - Over 80% of gastroenteritis in UK: *Campylobacter jejuni*
  - Around 10% of gastroenteritis in UK: *Campylobacter coli*
- Huge economic burden: Cost to the UK economy alone around 0.71 billion per year

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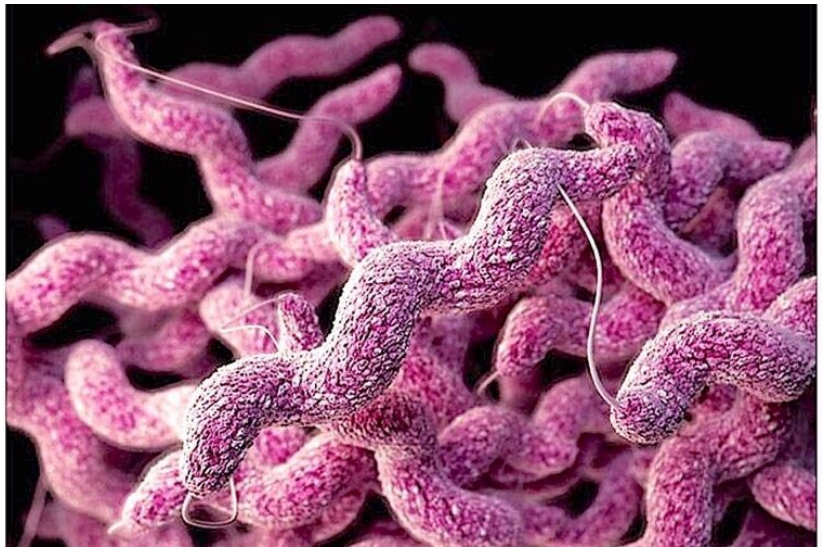

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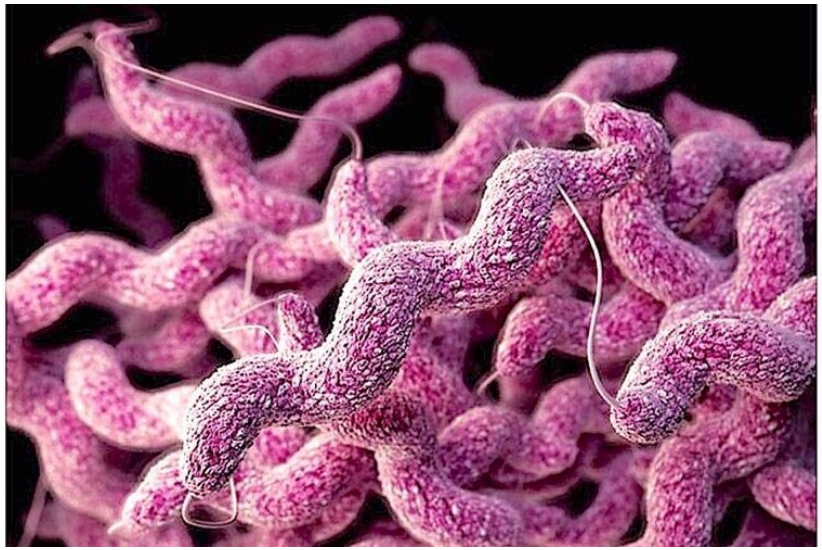

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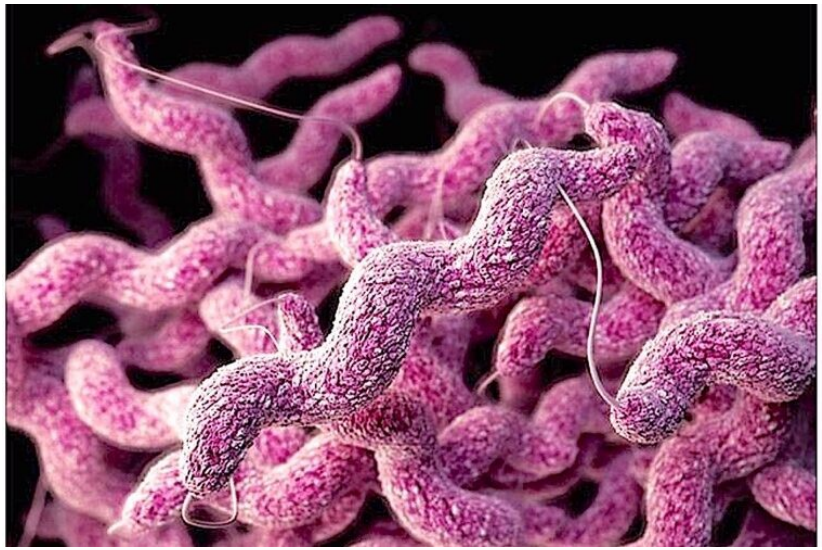

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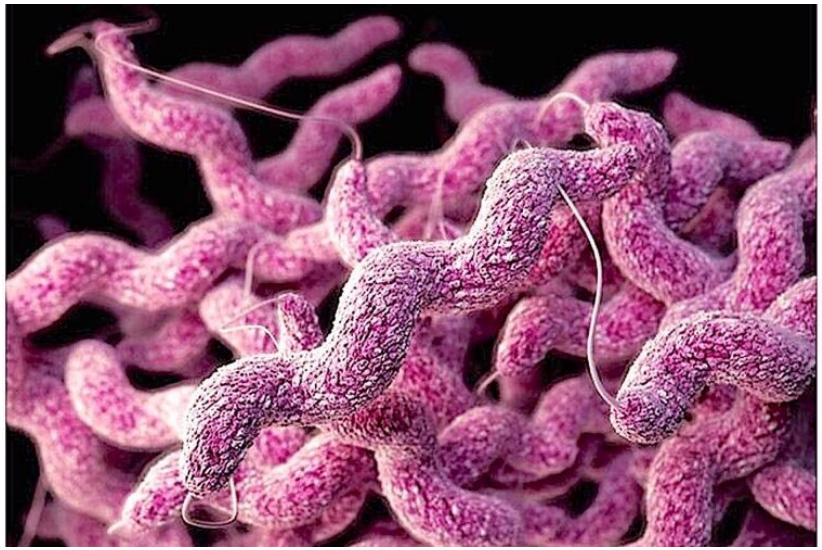

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Campylobacteriosis: causes, symptoms and treatment

- **Causes of infection**:
  - Mainly consumption of undercooked or poultry
  - But, also other contaminated food such as milk, water and other meat products
- **Symptoms**:
  - Diarrhoea, fever, nausea, vomiting, abdominal pain
  - Though, can cause long-lasting sequelae such as reactive arthritis, colitis, Guillain-Barré syndromes
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Campylobacter: growth and survival

- *Campylobacter* is a fastidious organism to grow under laboratory conditions
- Preferred growth requirements includes:
  - Temperature over 30°C
  - Microaerophilic conditions: a gas mix of 5-10% O_2, 5-10% CO_2 and 80-85% N_2 (some even using H_2)
  - Susceptible to environment and food processing stress
- These properties theoretically make it unsuitable for survival in natural environment or in food chain
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- Most *Campylobacter jejuni* isolates lack transporters for many small carbohydrates including glucose
- Lacks enzymes, glucokinase and phosphofructokinase, in glycolysis
- Lacks oxidative branch of Pentose Phosphate Pathway
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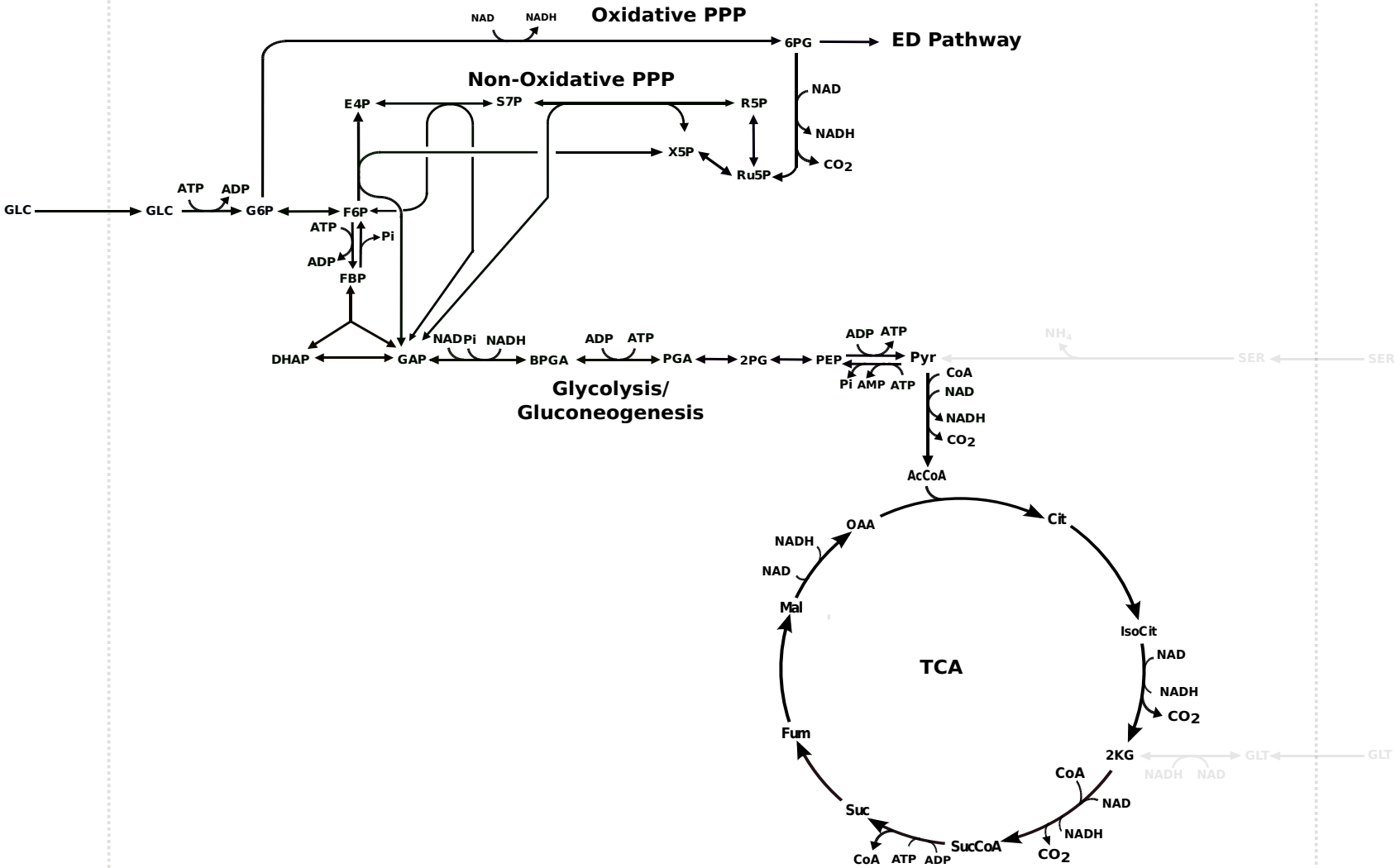
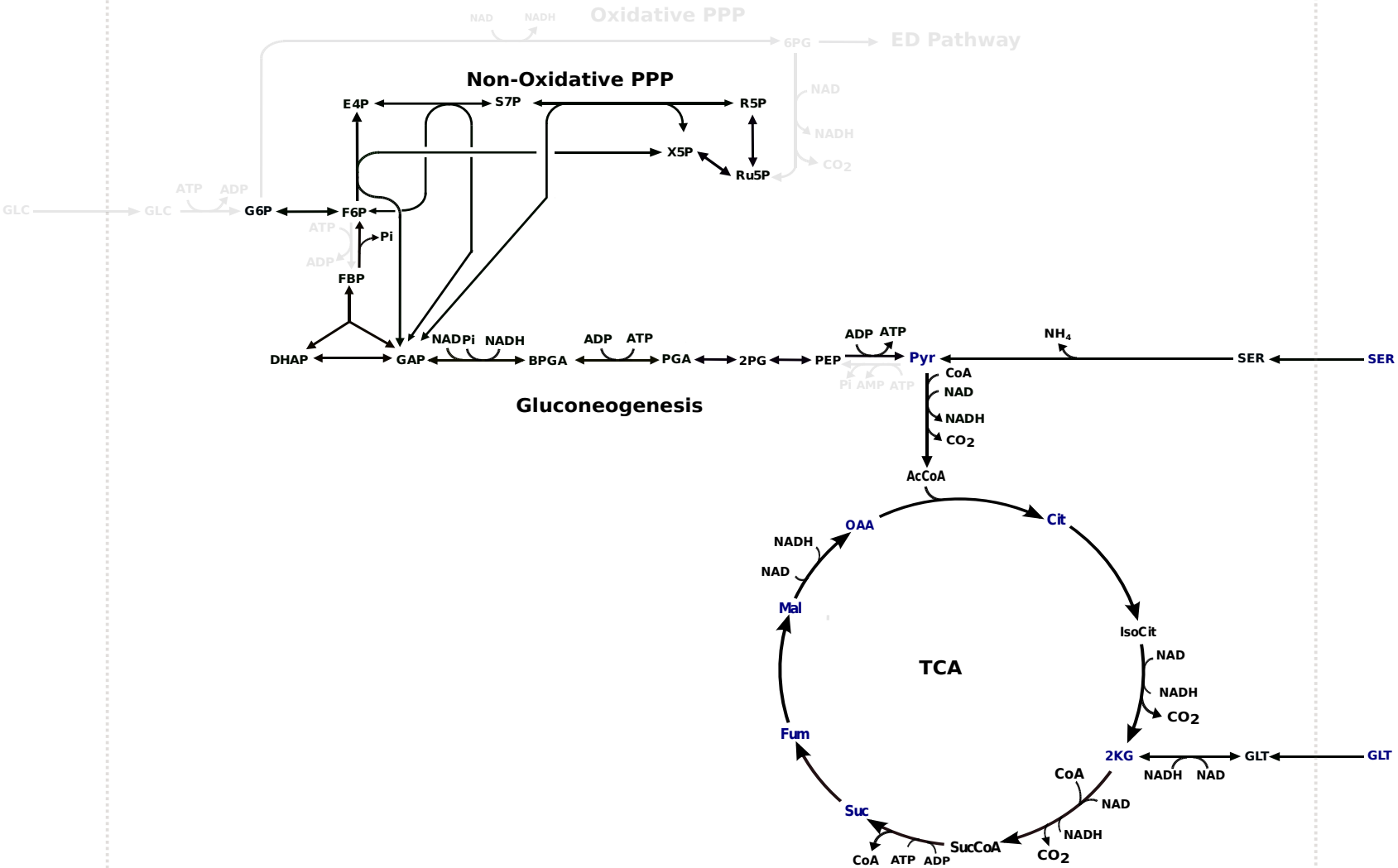
Central metabolism: *E. coli*



Central metabolism: *C. jejuni M1cam*



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Campylobacter jejuni isolates encode enzyme for a branched electron transport chain (ETC) and can utilise number of electron donors and acceptors

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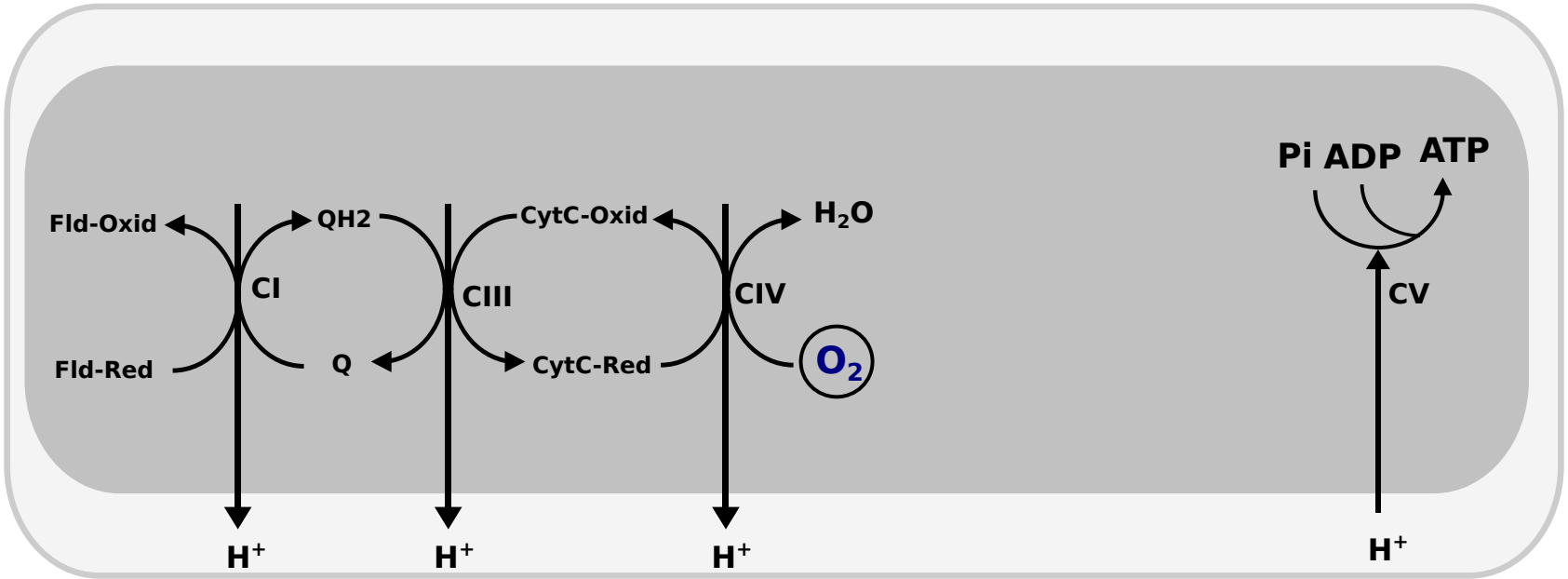
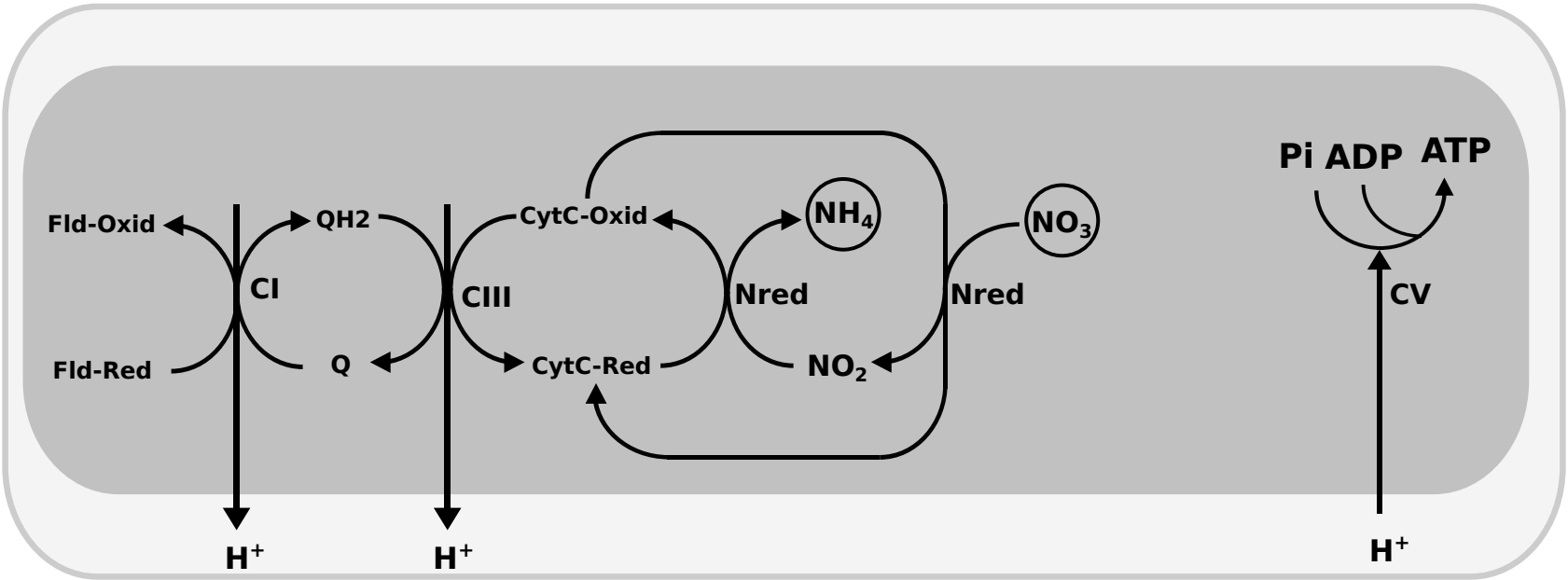
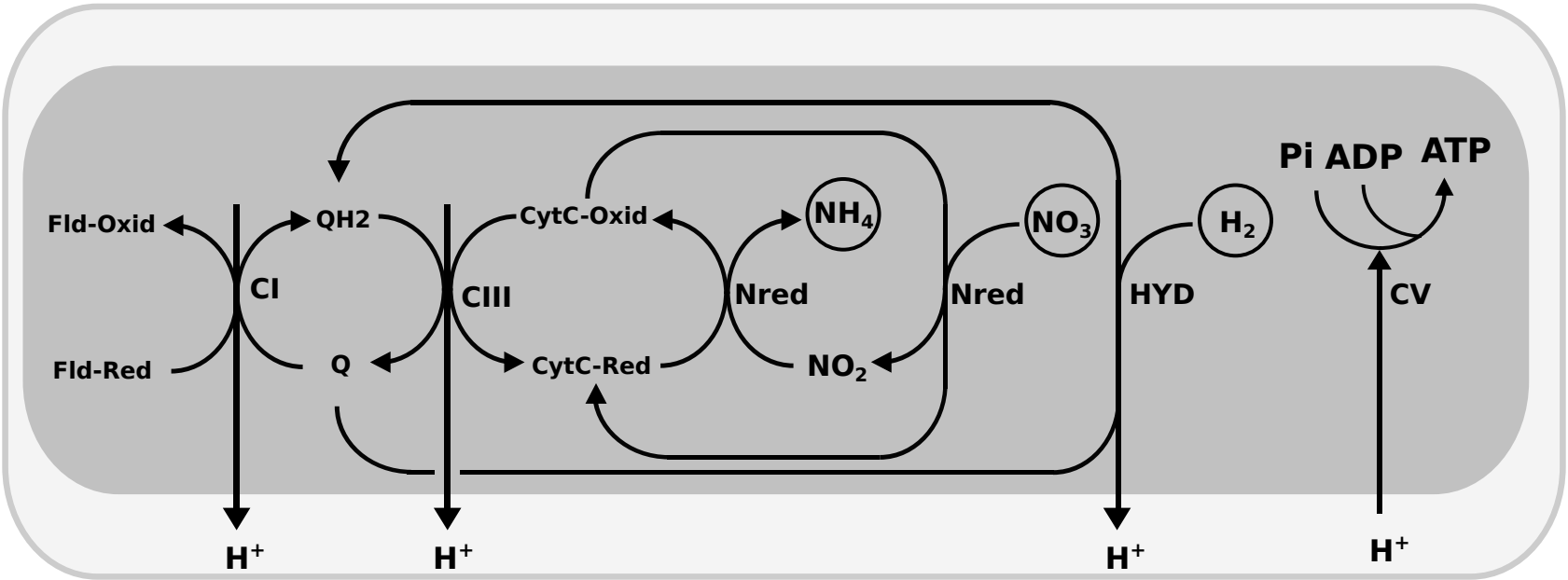


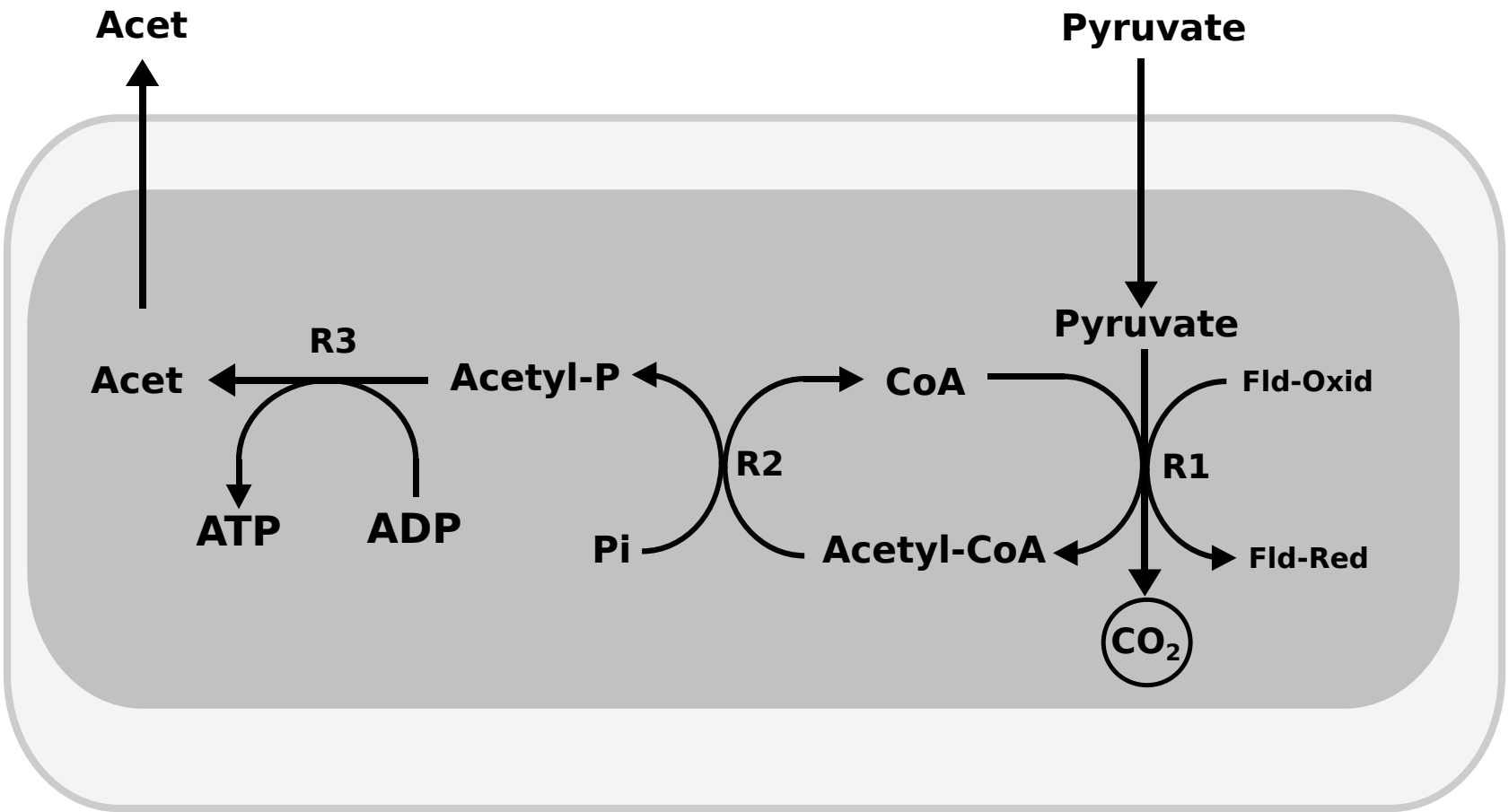
Anaerobic respiration: NO_3 as terminal electron acceptor



Hydrogen as electron donor



Substrate-level Phosphorylation



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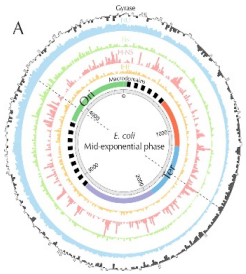
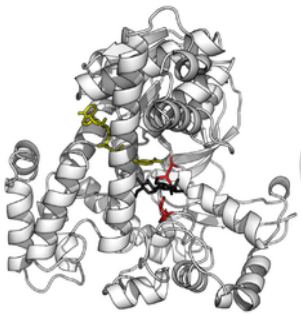
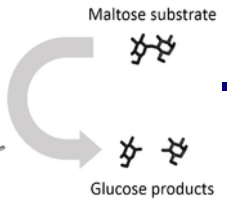
Genome-Scale Metabolic Model

GSM describes the metabolic interactions in a given organism based on reaction network predicted from enzymes encoded by the genome

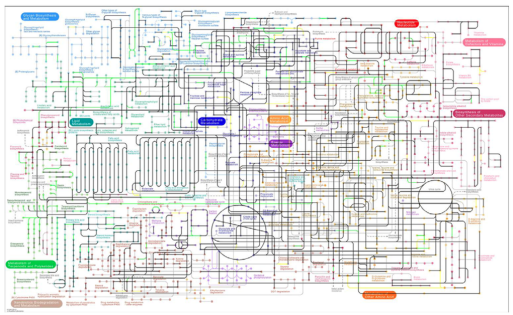


Genome Sequence



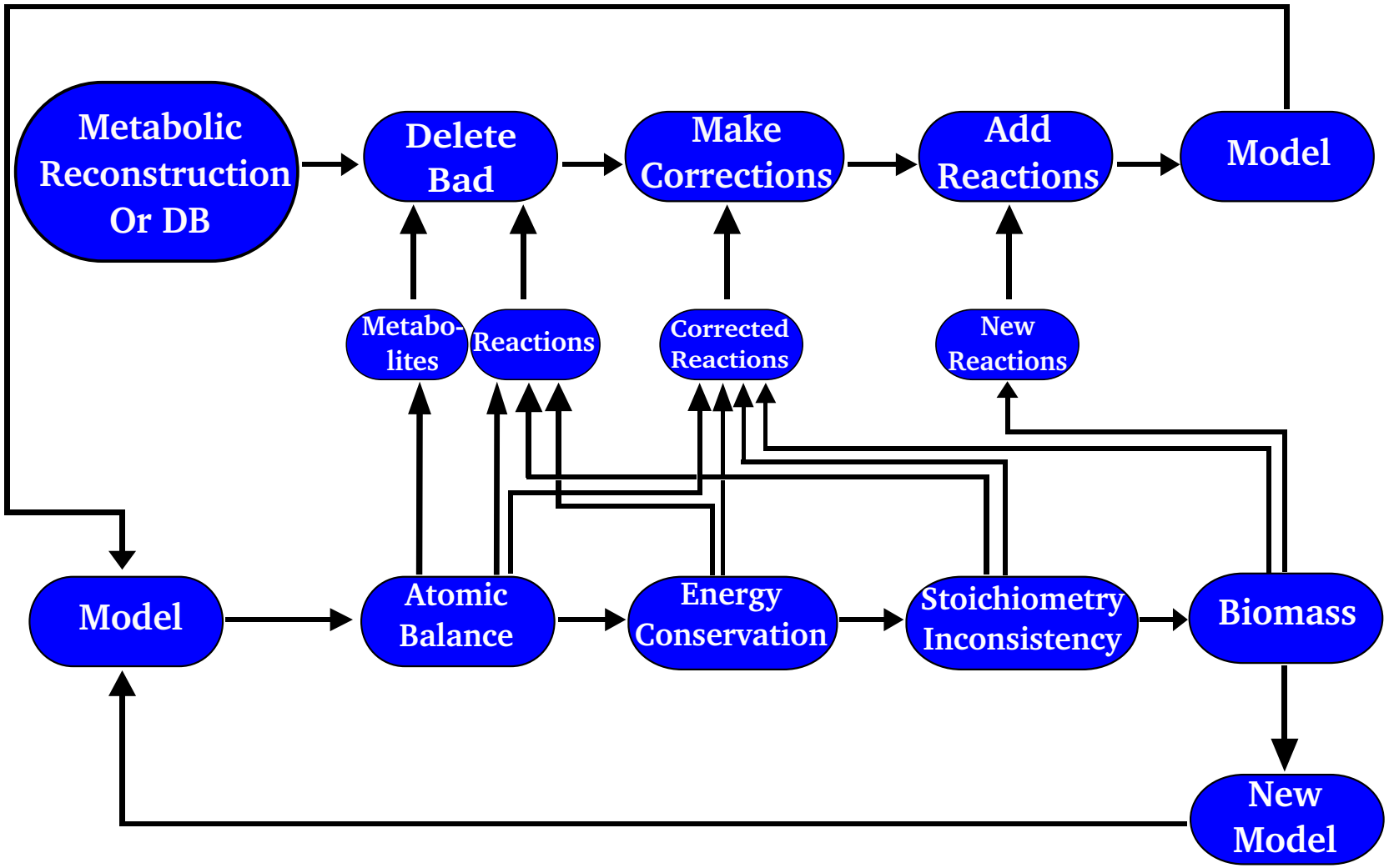


Gene prediction and annotation
(Prodigal and Prokka)

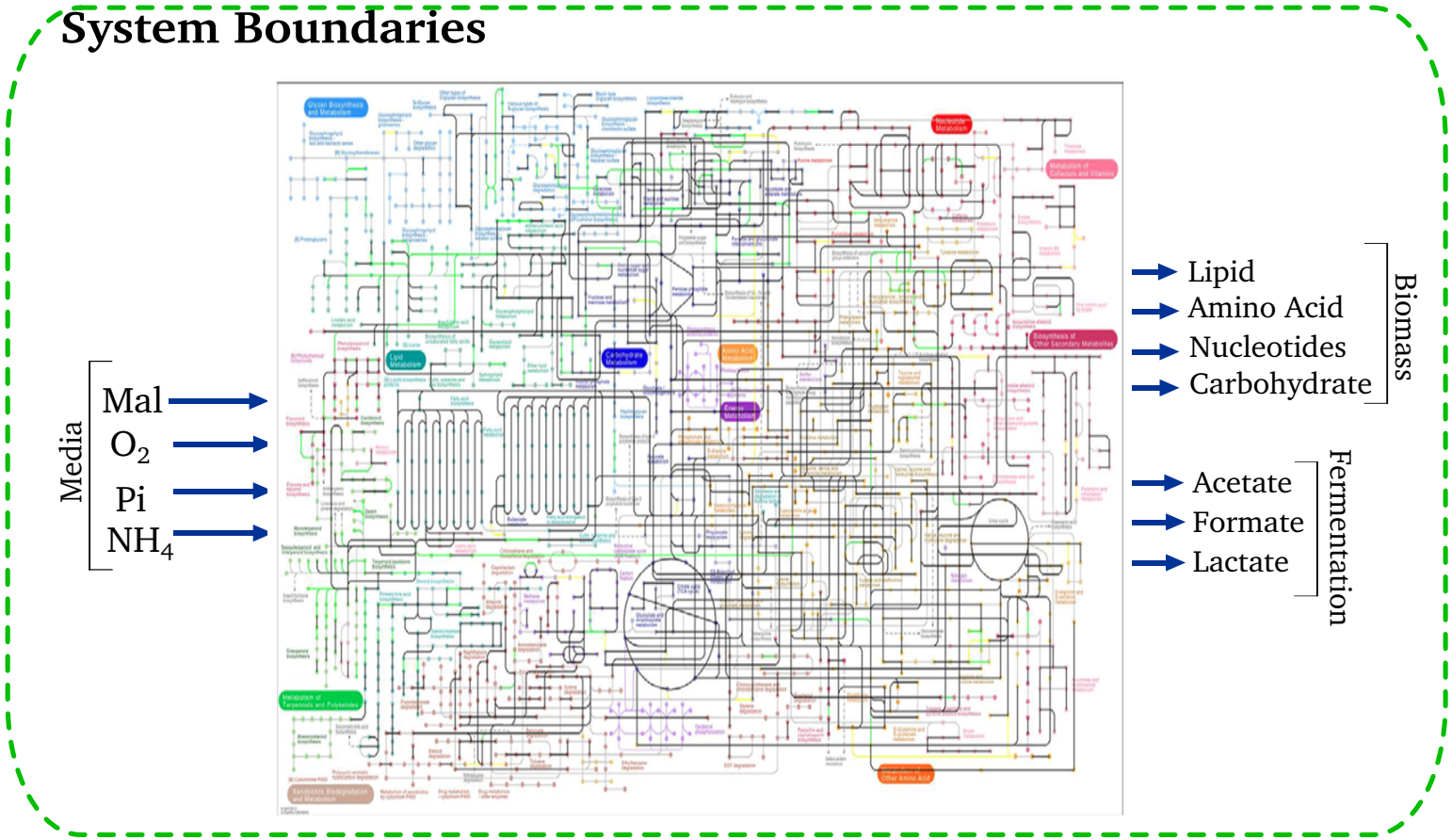


Metabolic Reconstruction
(Pathway Tools)

Genome-Scale Metabolic Model Construction Pipeline



Genome-Scale Metabolic Model



Genome-scale metabolic model of *C. jejuni M1cam*

- The GSM consists of 992 reactions excluding transporters and 967 internal metabolites
- There are 51 biomass transporters, 41 media transporters, 10 by-product transporters
- Model is curated and validated for mass and energy conservation
- Model is free of stoichiometric inconsistencies

Check the numbers for your model!!!

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Growth Media

Clinical labs use rich media (Bolton broth)
Academic labs use rich media (Brucella broth)

rich undefined media

Ingredients	Grams/Litre
Casein Enzymic Hydrolysate	10.0
Peptic Digest of Animal Tissue	10.0
Yeast Extract	2.0
Dextrose	1.0

MCLMAN media: defined minimal media for *C. jejuni* strains based on NCTC11168, Alazzam *et al. Res. in Microbiol.*

Fitnesses of *C. jejuni* strains in MCLMAN medium.

Fitness[a]	*C. jejuni* strains
++	NCTC 11168, 81-176, BKR314, AKR6, Cj480, AKR672, E6-B1, BKR266, E12-K3, MJL640, BKR589, BKR290, BKR522
+	F38011, BKR646, BKR293, BKR146
–	AKR546, BKR645, BKR396,A728, Bf, A922, A928

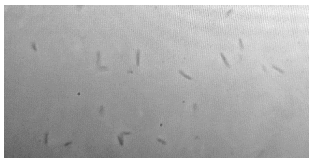
(++): $W > 0.85$, (+): $0.85 \leq W \leq 0.25$, (–): $W < 0.25$.
[a] Relative fitness indexes were obtained using NCTC 11168 as reference.

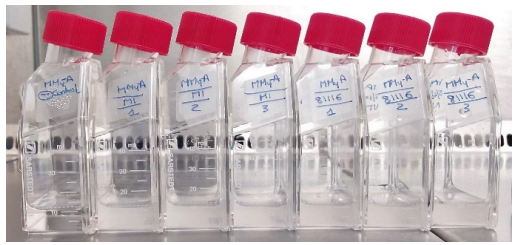
Tested with M1cam and 81116
Positive results only in 81116

Suggests difference in substrate auxotrophy

Silac DMEM/Ham's F-12 media

- *Used for:* Mammalian cell culture
- Defined but **Not minimal**: reducing list of ingredients necessary





Positive results
M1cam and 81116

OD of culture (600nm) M1cam = 0.34 ± 0.04

OD of culture (600nm) M1cam in synthetic DMEM/F12 = 0.34 ± 0.04

Model analysis on DMEM media for biomass production

GSM was analysed on DMEM media where the objective function is to minimize the sum of all (absolute) flux values (including transporters) with constraints as described below

$$\begin{aligned} &\text{minimise} \quad : \sum_{i=1}^{n} v_i \quad \text{objective function} \\ &\text{constraints} \begin{cases} \mathbf{N} \cdot \mathbf{v} = \mathbf{0} \text{ steady state constraint} \\ v_{i..j} = b_{i..j} \text{ biomass constraint} \\ v_{\text{ATPase}} = \text{A cell maintenance cost} \\ v_{Otx} \leq \text{O microaerophilic constraint} \end{cases} \end{aligned} \tag{1}$$

Solving eq 1 return an LP solution i.e GSM is able to produce all biomass components under given condition in DMEM media.

How many reactions are present in your LP solution!!!

Model analysis for identification of substrate auxotrophy

Auxotrophy definition: the inability of an organism to synthesize a particular organic compound required for its growth and therefore, relies on external source such as media, host

- Substrate auxotrophy, in the GSM, was determined by repeatedly attempting to solve eq 1 with individual media transporters set to zero, one at a time
- Failure to obtain a solution was taken as demonstrating auxotrophy with respect to the component whose transporter was thus constrained

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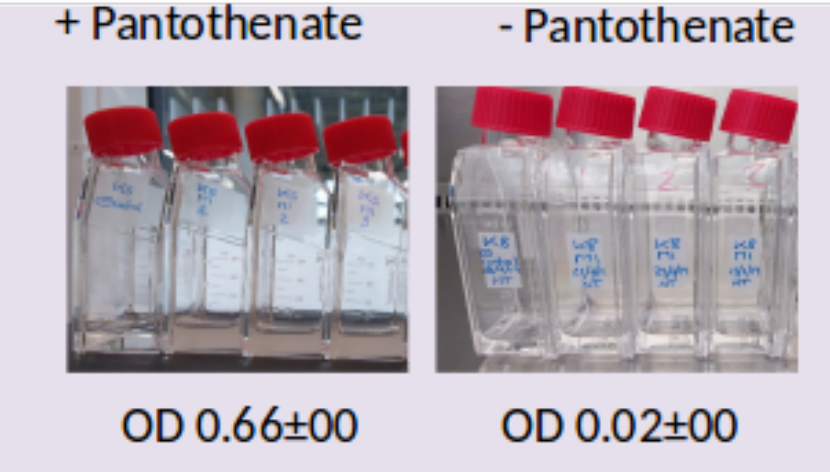
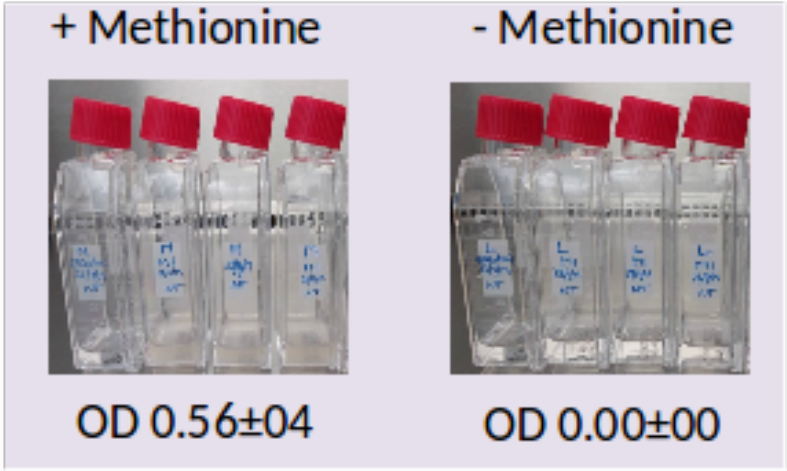
Identification of substrate auxotrophy

Model Results: *Campylobacter jejuni* M1cam is auxotrophic to **pantothenate** and **methionine** i.e removing any of these substrate would support no growth/biomass production

Experimental Result/Validation





In addition, *C. jejuni* M1cam is also auxotrophic to niacinamide.

Which substrates are auxotrophic in your model?

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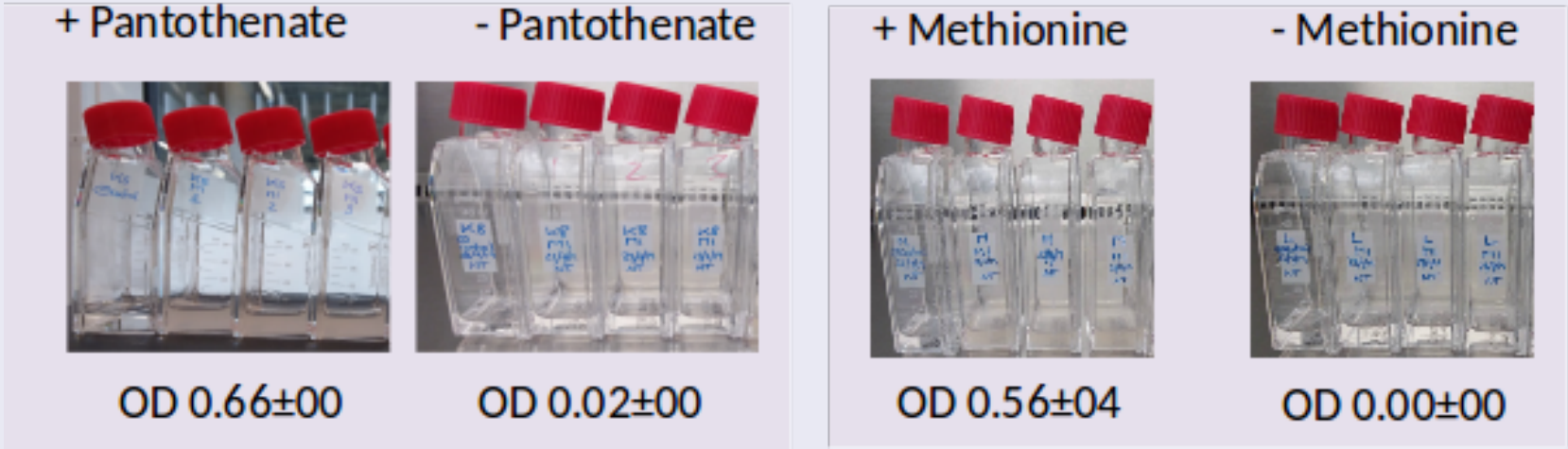

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Which substrates are auxotrophic in your model?

Amino acids/Pyruvate preference

- media import flux = biomass export flux, suggests their direct incorporation into the biomass protein component
- media import flux > biomass export flux (aspartate, cysteine, serine, glutamine, proline, and pyruvate)

Substrates	GSM	Experiment
Serine	Preferred	Growth promoting
Glutamine	Preferred	Growth promoting
Glutamate	Not preferred	Growth promoting
Proline	Preferred	No effect on growth
Aspartate	Preferred	No effect on growth
Cysteine	Preferred	Growth promoting
Pyruvate	Preferred	Growth promoting

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Campylobacter jejuni M1cam specific defined growth media

Substrates	DMEM/ F-12*	MM1	MM2	MM3	MM4
Auxotropic					
Methionine	115.7 μM	4 mM	4mM	8 mM	6.8 mM
Pantothenate	4.7 μM	9.4 μM	9.4 μM	9.4μM	9.4 μM
Niacinamide	16.6 μM	33.1 μM	33.1 μM	33.1 μM	33.1 μM
Carbon source					
Pyruvate	0.5 mM	10 mM	10 mM	10 mM	10 mM
Growth-improving					
Cysteine·HCl	100 μM	4 mM	4 mM	–	4 mM
Serine	250 μM	10 mM	10 mM	10 mM	–
Glutamine	2.5 mM	10 mM	–	10 mM	13.6
Glutamate	50 μM	–	20 mM	–	–
Inorganic salts	same as DMEM/F-12 (**Table S1**)				
Growth (OD 600)					
24 h	0.38 ± 0.01	0.31 ± 0.05	0.14 ± 0.03	0.02 ± 0.01	0.15 ± 0.01
48 h	0.34 ± 0.04	1.05 ± 0.06	1.07 ± 0.09	0.41 ± 0.04	0.57 ± 0.01

OD results expressed as mean ± stdev (n = 3).
**Table S1 for all the components of DMEM/F-12.*

Tejera et al. Genome-Scale Metabolic Model Driven Design of a Defined Medium for *Campylobacter jejuni* M1cam. 2020. Front. Microbiol. 11:1072.

Summary: Growth media design

Using an integrated metabolic modelling and growth experiment, we have

- identified auxotrophic and growth promoting substrates for *Campylobacter jejuni* M1cam
- designed defined minimal media (with only 8 organic substrates compared to over 30 in DMEM)
- improved growth

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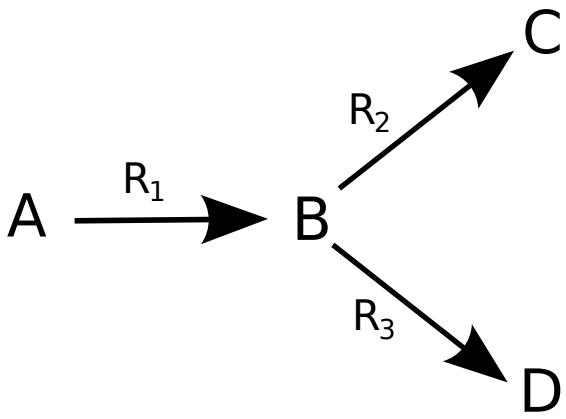
Reaction Network to Mathematical Object

Hypothetical network



Stoichiometric matrix

$$\mathbf{N} = \begin{array}{c} \begin{matrix} \mathbf{R_1} & \mathbf{R_2} & \mathbf{R_3} \end{matrix} \\ \begin{bmatrix} -1 & 0 & 0 \\ 1 & -1 & -1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \end{array} \begin{matrix} \mathbf{A} \\ \mathbf{B} \\ \mathbf{C} \\ \mathbf{D} \end{matrix}$$

Rate of change of metabolites:

$$\begin{aligned} dA/dt &= -v_{R1} \\ dB/dt &= v_{R1} - v_{R2} - v_{R3} \\ dC/dt &= v_{R2} \\ dD/dt &= v_{R3} \end{aligned}$$

Matrix representation

$$\begin{bmatrix} \mathbf{dA/dt} \\ \mathbf{dB/dt} \\ \mathbf{dC/dt} \\ \mathbf{dD/dt} \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 \\ 1 & -1 & -1 \\ 0 & 1 & 0 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} \mathbf{v_{R1}} \\ \mathbf{v_{R2}} \\ \mathbf{v_{R3}} \end{bmatrix}$$

Linear Programming: Flux Balance Analysis (FBA)

FBA is a linear programming (LP) based approach to assign fluxes to reactions based on the law of mass conservation.

Optimises (minimise or maximise) reaction fluxes subject to:

- Steady state constraint
- Additional flux constraints such as such as thermodynamics constraints, demand for biomass production, limits on reaction rates

$$\text{minimise}|\text{maximise} \quad : \quad \mathbf{v}_{targs} \qquad \text{objective function}$$

$$\text{constraints} \quad \begin{cases} \mathbf{N} \cdot \mathbf{v} = \mathbf{0} & \text{steady-state constraint} \\ max_i \geq v_i \geq min_i & \text{media constraint} \\ v_{i..j} \geq t_{i..j} & \text{biomass constraint} \\ \mathbf{v}_{\text{ATPase}} = ATPase & \text{cell maintenance cost} \end{cases}$$