Determination of minimal media requirements for *Campylobacter jejuni*

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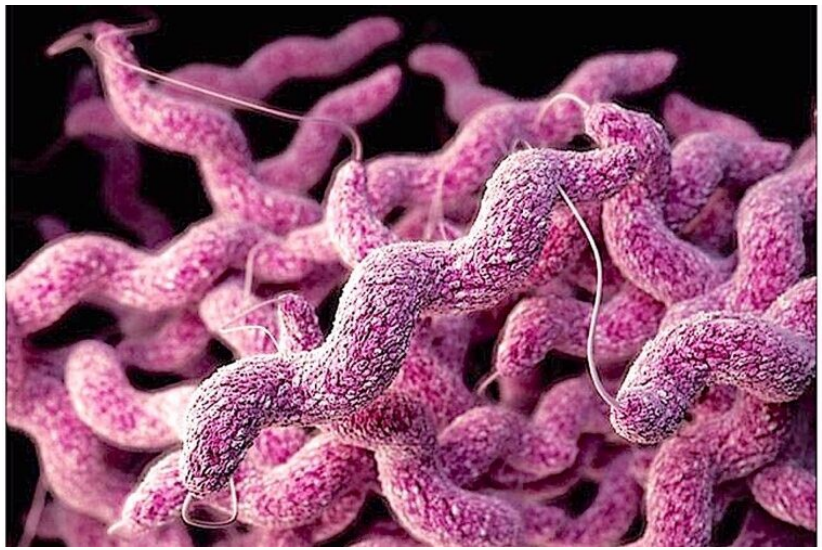
Norwich, UK

September 26, 2024

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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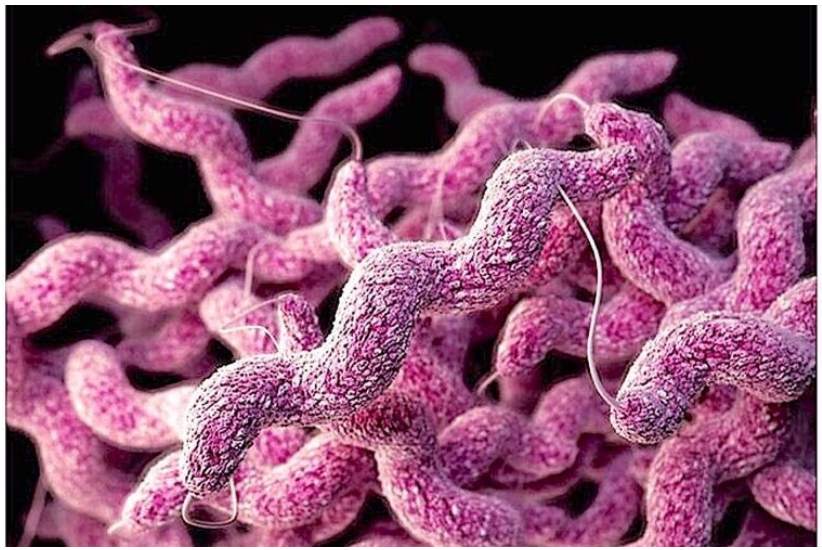

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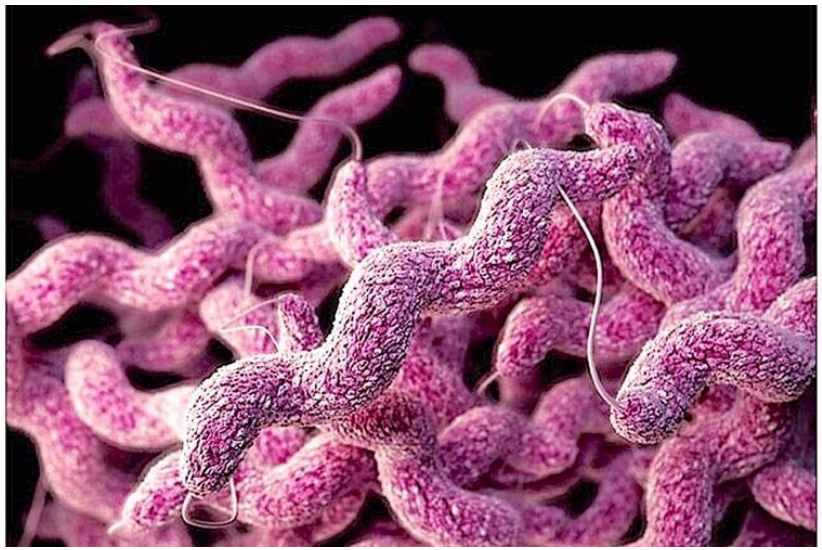

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Campylobacteriosis: causes, symptoms and treatment

- **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
- **Treatment**:
  - Usually symptoms only last between 2 to 5 days
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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
- **However,** it is widely spread in environment and can be readily isolated from food, water, poultry litter, milk
- **Therefore, studying the growth and survival requirement of Campylobacter is important for the intervention**

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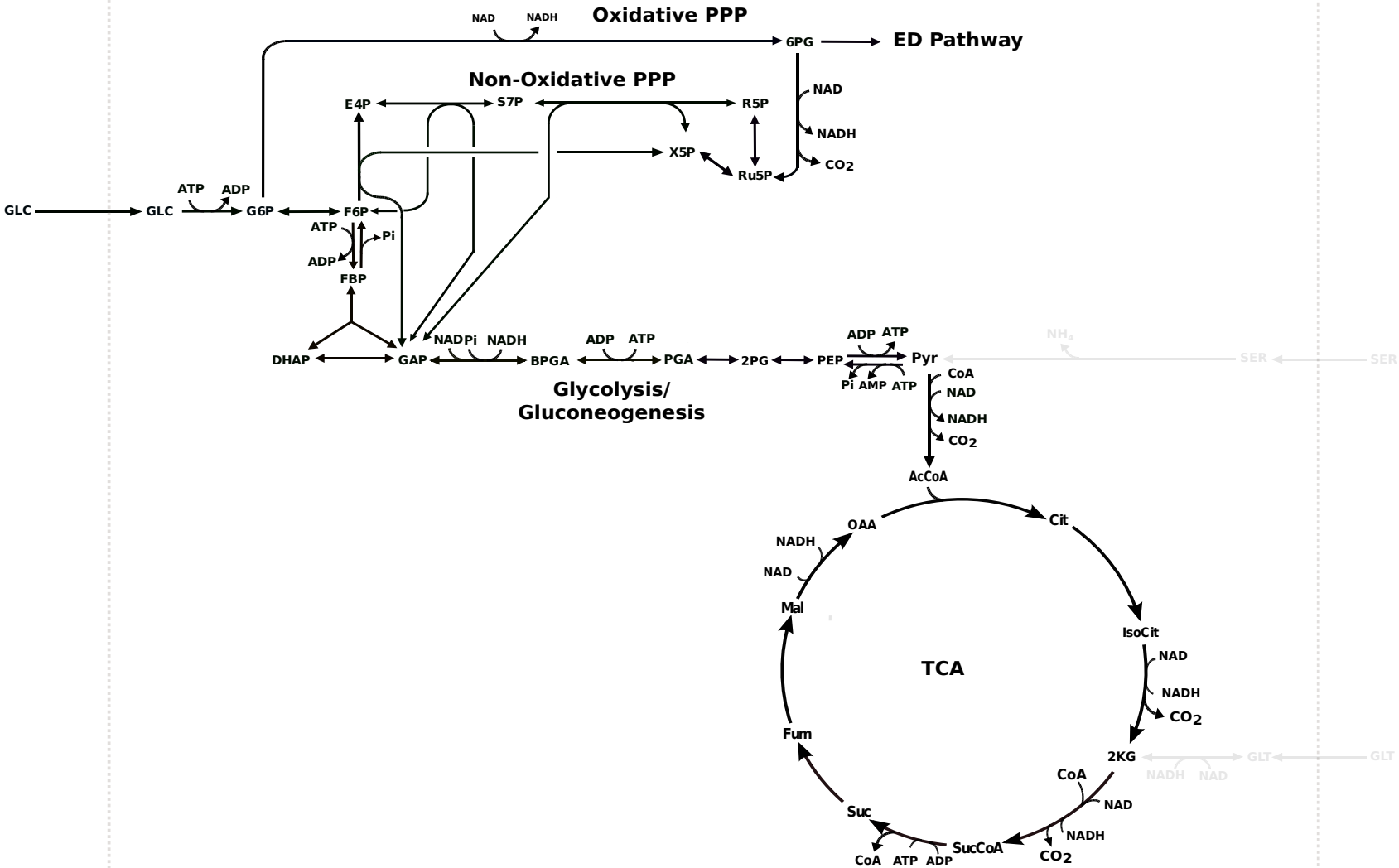
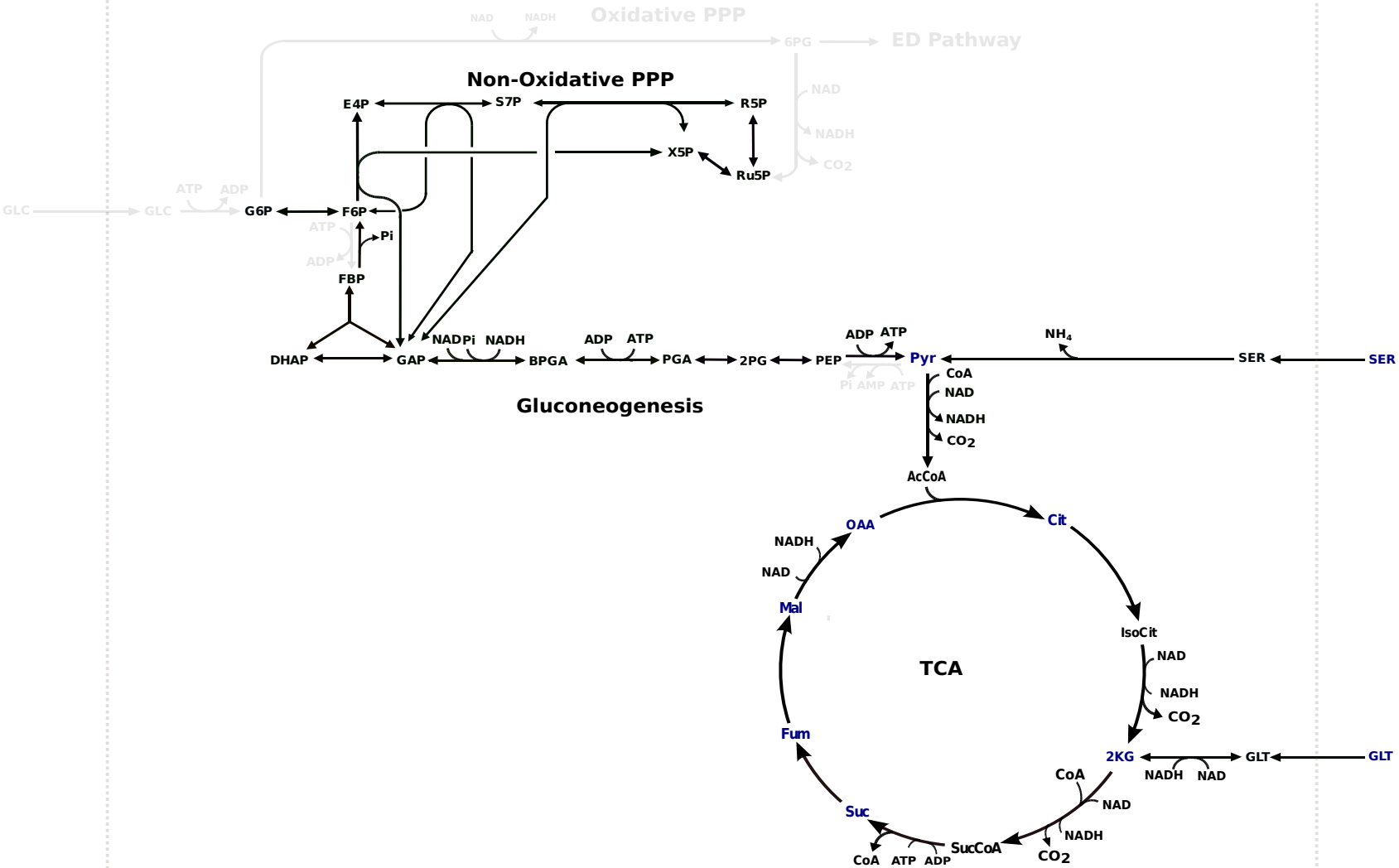
Metabolism: *Campylobacter jejuni*

- 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
- Preferred carbon and energy sources have been reported to be mainly amino acids and tricarboxylic acid (TCA) cycle intermediates such as serine, pyruvate and succinate

Central metabolism: *E. coli*



Central metabolism: *C. jejuni M1cam*

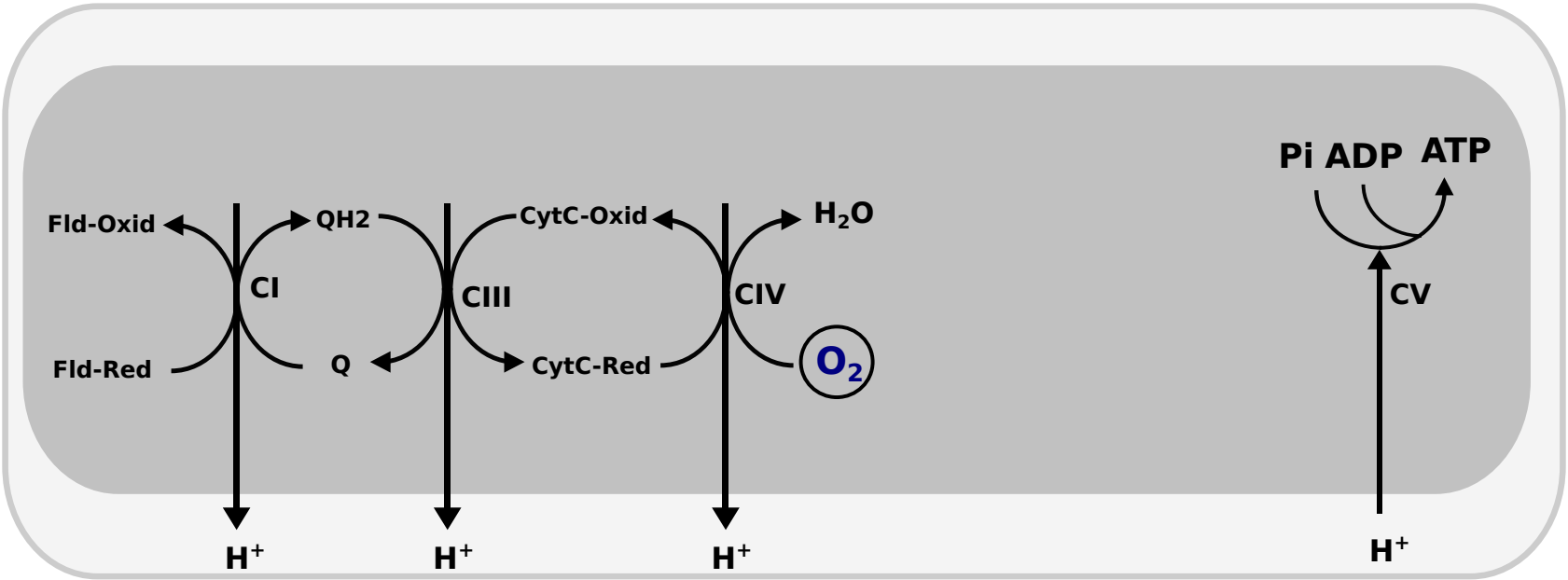


Energy metabolism: *Campylobacter jejuni*

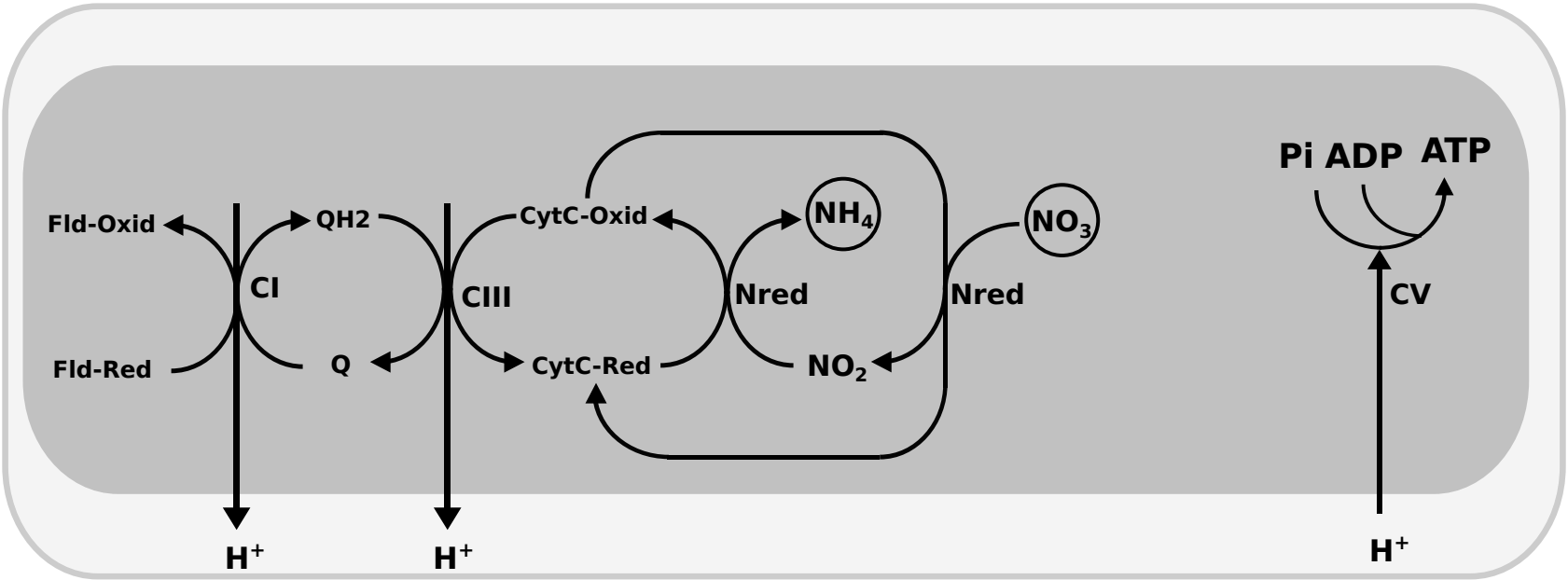
Campylobacter jejuni isolates encode enzyme for a branched electron transport chain (ETC) and can utilise number of electron donors and acceptors

- Aerobic respiration: utilise O_2 as terminal electron acceptors
- Anaerobic respiration: utilise electron acceptors other than O_2 such as nitrate, fumarate
- Substrate-level phosphorylation through acetate kinase
- Genome also encodes for periplasmic hydrogenase reaction that can utilise H_2 as electron donor
- **We will see some of these during our practical session!!!**

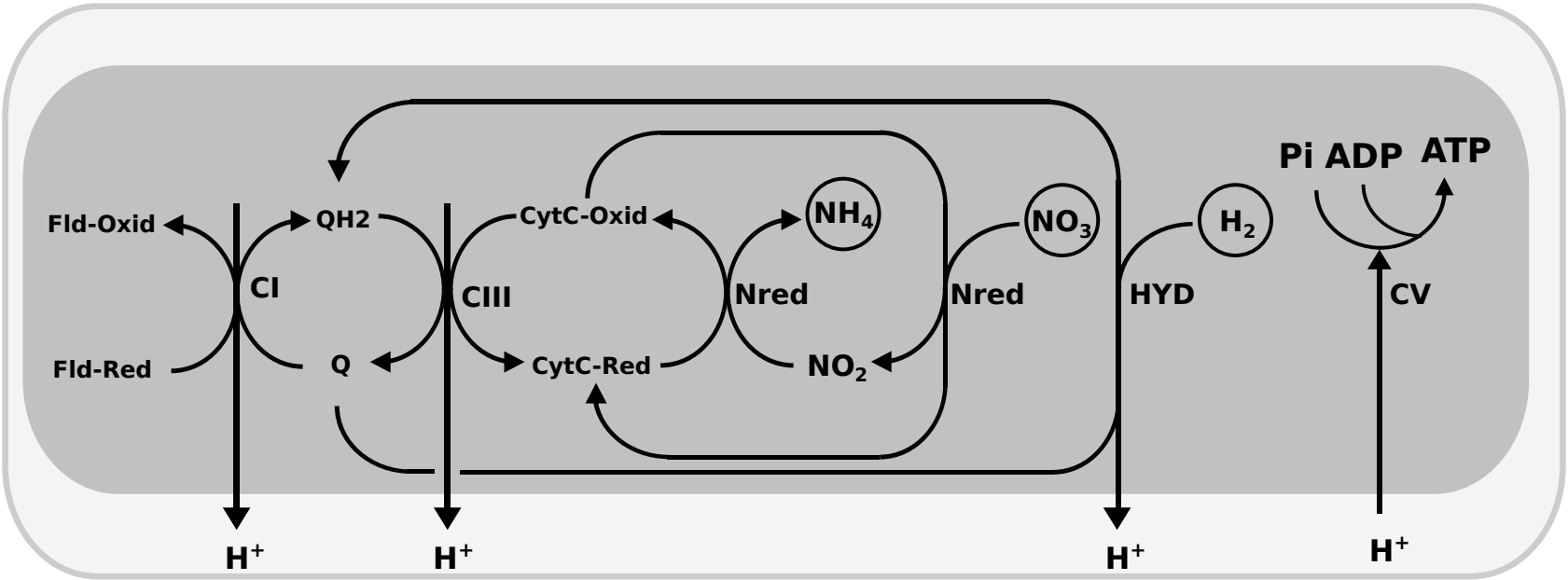
Aerobic respiration: O_2 as terminal electron acceptor



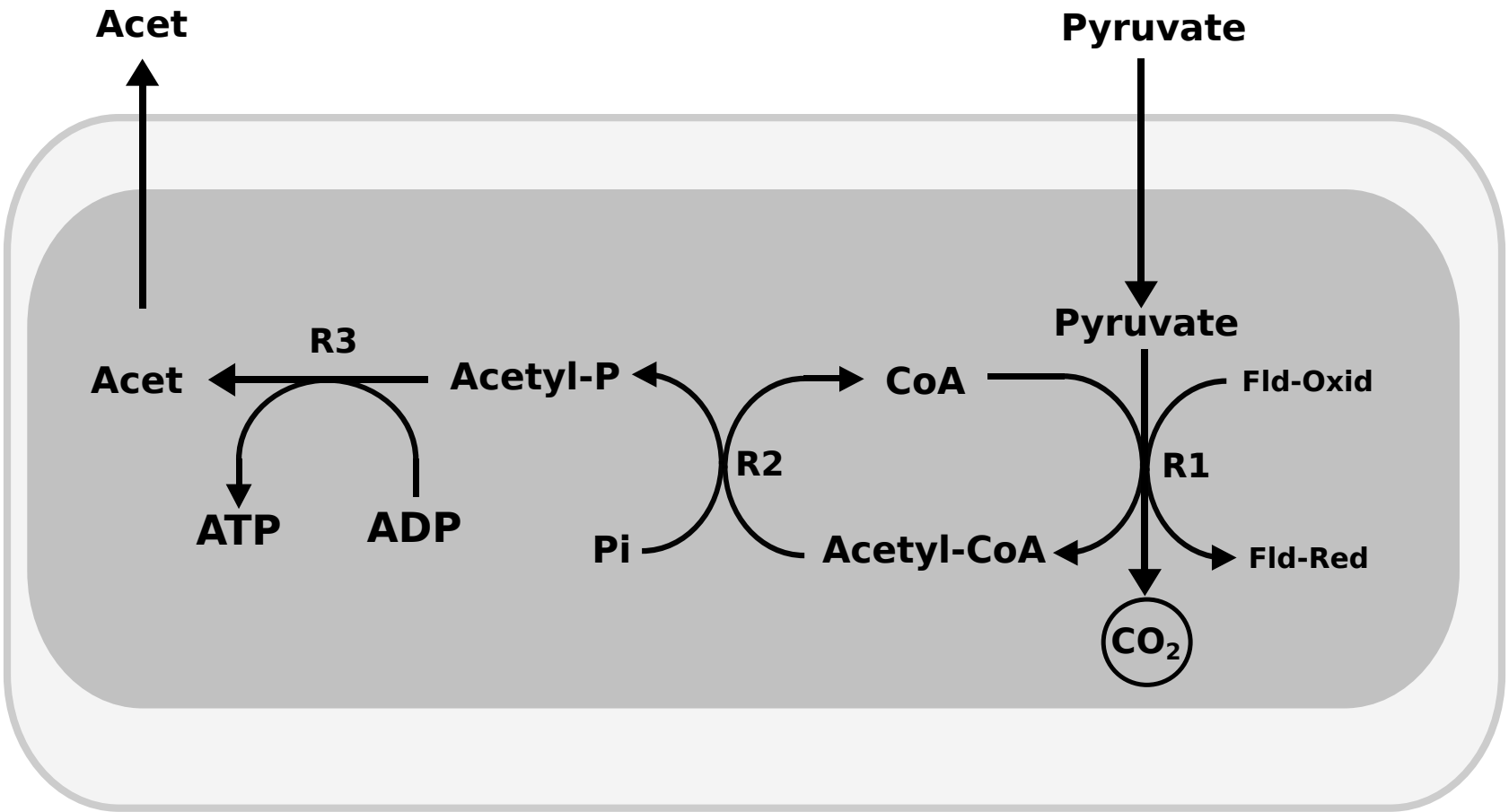
Anaerobic respiration: NO_3 as terminal electron acceptor



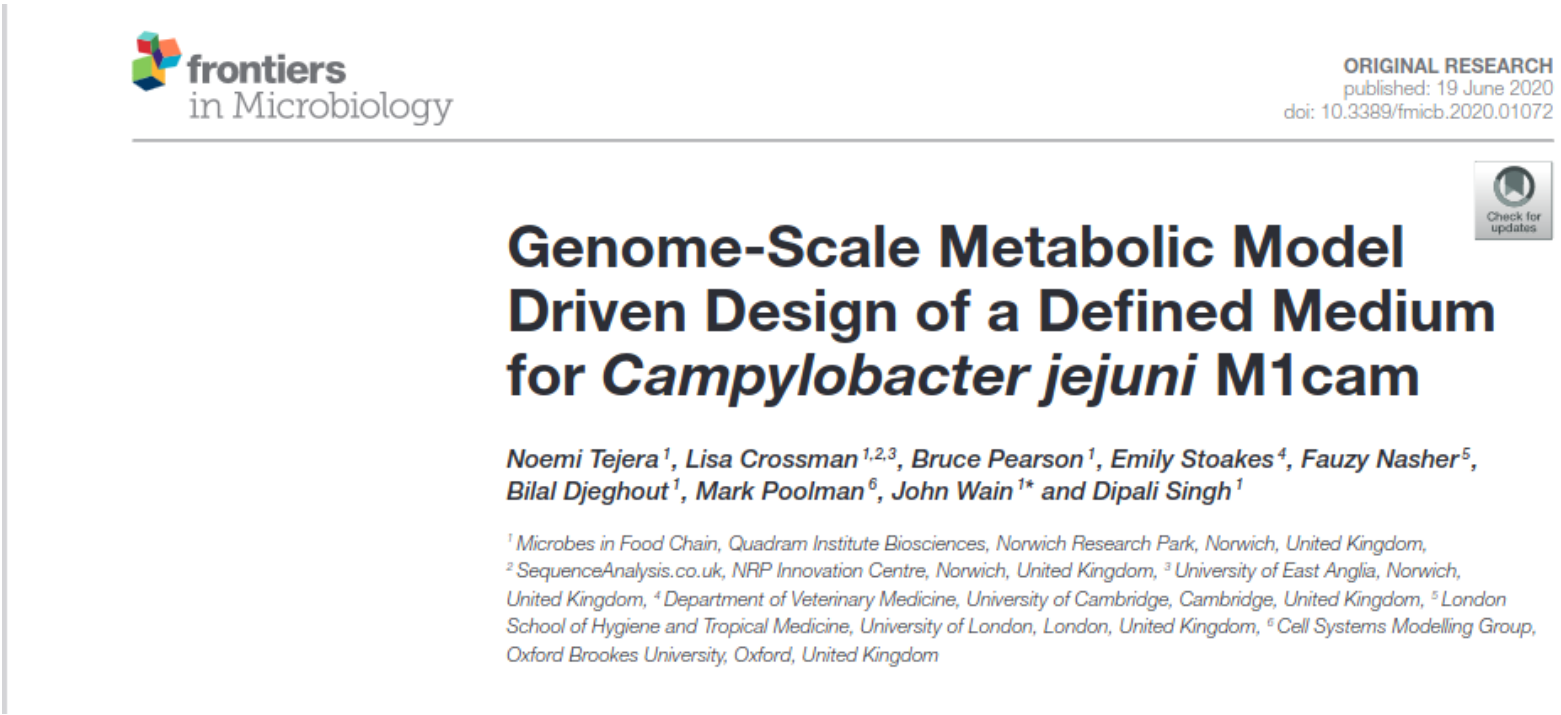
Hydrogen as electron donor



Substrate-level Phosphorylation



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



frontiers in Microbiology

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Check for updates

Genome-Scale Metabolic Model Driven Design of a Defined Medium for *Campylobacter jejuni* M1cam

Noemi Tejera[1], Lisa Crossman[1,2,3], Bruce Pearson[1], Emily Stoakes[4], Fauzy Nasher[5], Bilal Djeghout[1], Mark Poolman[6], John Wain[1] and Dipali Singh[1]*

[1] Microbes in Food Chain, Quadram Institute Biosciences, Norwich Research Park, Norwich, United Kingdom, [2] SequenceAnalysis.co.uk, NRP Innovation Centre, Norwich, United Kingdom, [3] University of East Anglia, Norwich, United Kingdom, [4] Department of Veterinary Medicine, University of Cambridge, Cambridge, United Kingdom, [5] London School of Hygiene and Tropical Medicine, University of London, London, United Kingdom, [6] Cell Systems Modelling Group, Oxford Brookes University, Oxford, United Kingdom*

- Identified auxotrophic and growth promoting substrates for *Campylobacter jejuni* M1cam
- Designed defined media for improved growth

Project aim: Design of minimal media

- **Aim:** Identify minimal growth requirement for *Campylobacter jejuni*, targeted strain M1cam:
  - isolated from human stool
  - sequenced and annotated
  - has transformed to generate a library of mutants
- **Methods:** Genome-scale metabolic model (GSM) and growth experiments
  - GSM was constructed, curated and validated
  - Flux Balance Analysis (FBA) was applied to identify substrate auxotrophies
  - GSM results were validated using growth experiments
- **Results:** Identified substrate auxotrophies and minimal growth requirement in *Campylobacter jejuni* M1cam

Tejera et al. 2020. Front. Microbiol. 11:1072.

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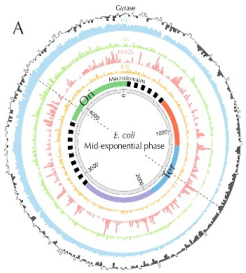
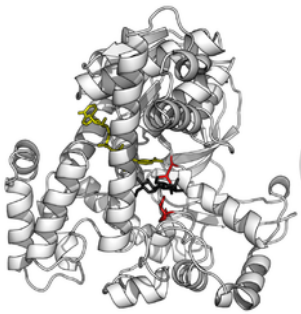
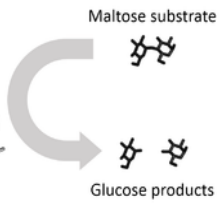
Genome-Scale Metabolic Model

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

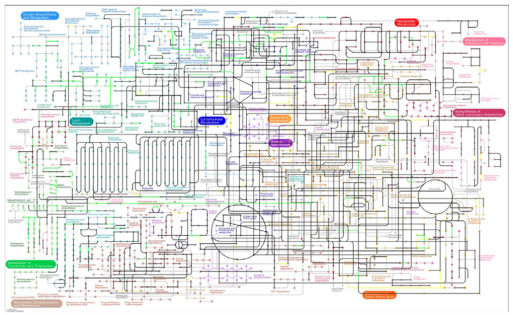


Genome Sequence



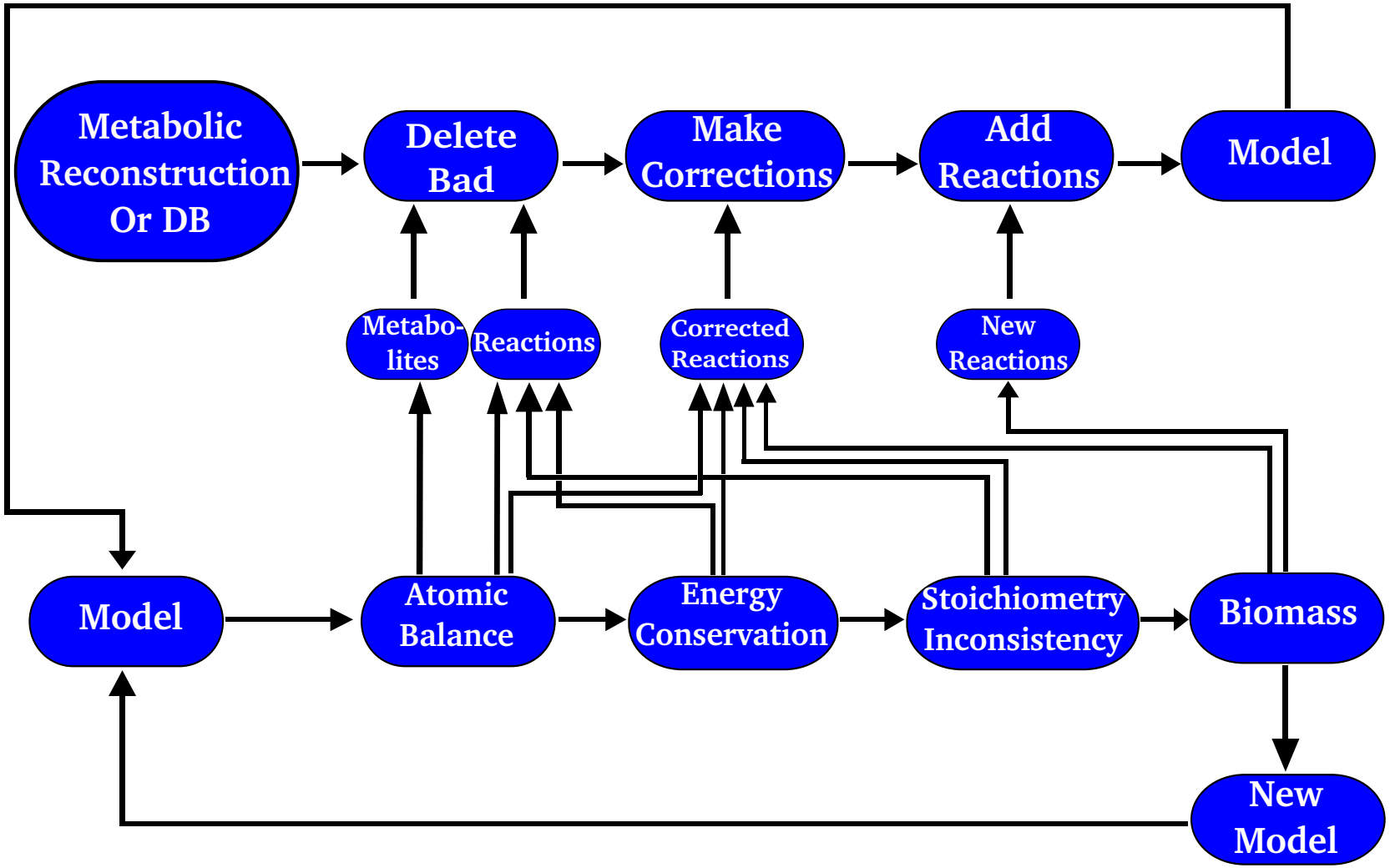


Gene prediction and annotation
(Prodigal and Prokka)



Metabolic Reconstruction
(Pathway Tools)

Genome-Scale Metabolic Model Construction Pipeline



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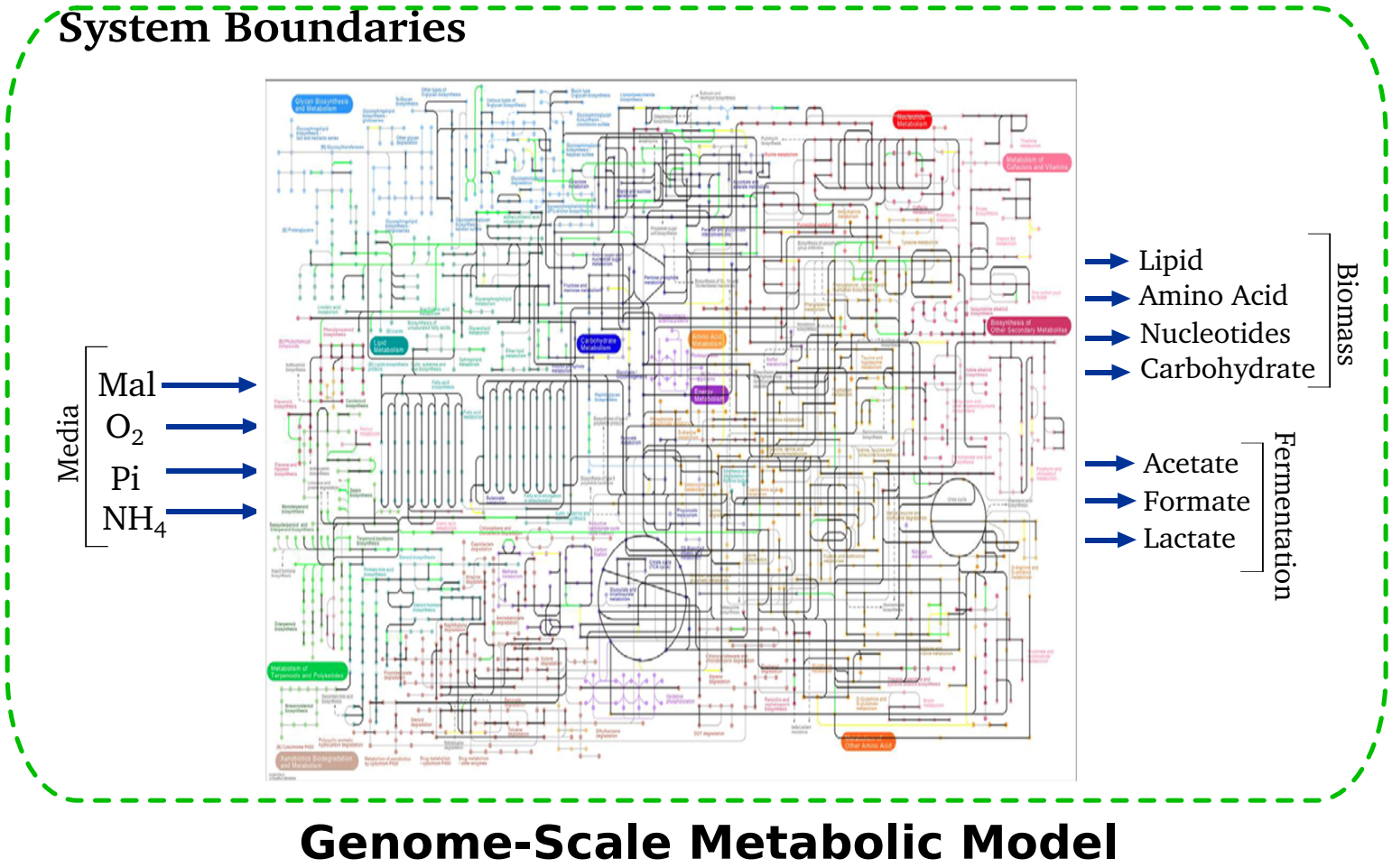


Genome-scale metabolic model of *C. jejuni M1cam*

- 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!!!

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

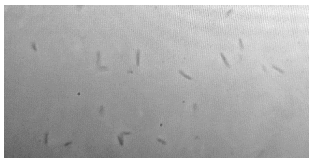
(++): $W > 0.85$, (+): $0.85 \leq W \leq 0.25$, (–): $W < 0.25$.
[a] Relative fitness indexes were obtained using NCTC 11168 as reference.

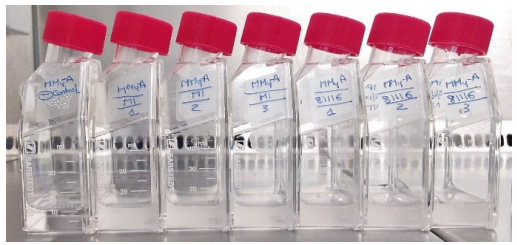
Tested with M1cam and 81116
Positive results only in 81116

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

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

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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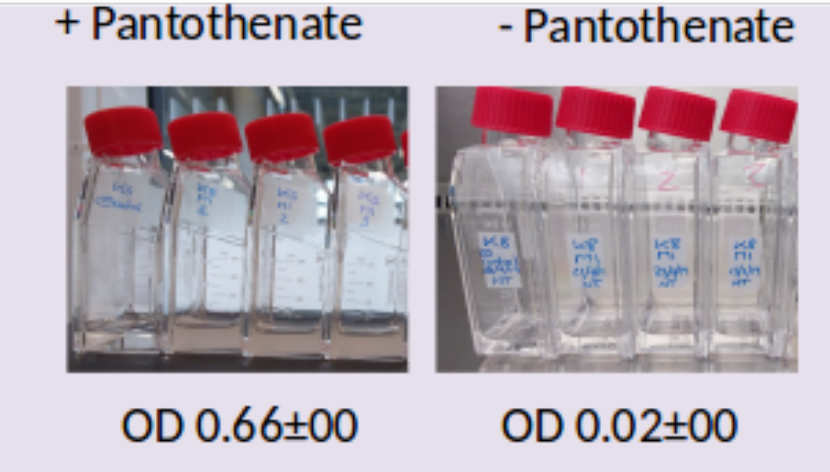
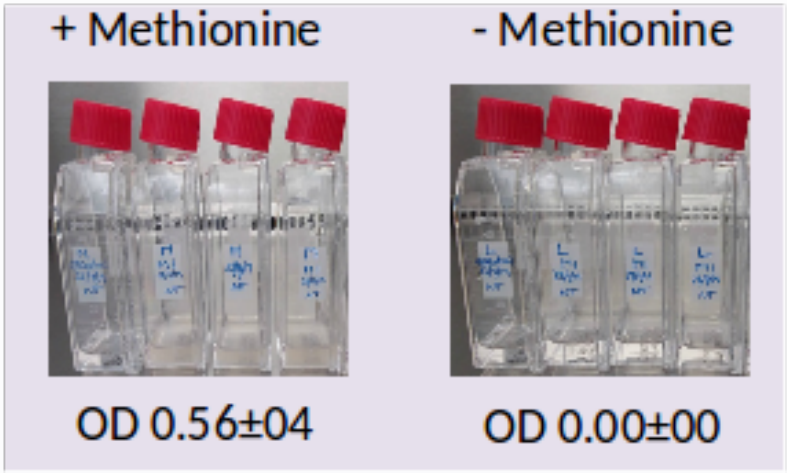
Identification of substrate auxotrophy

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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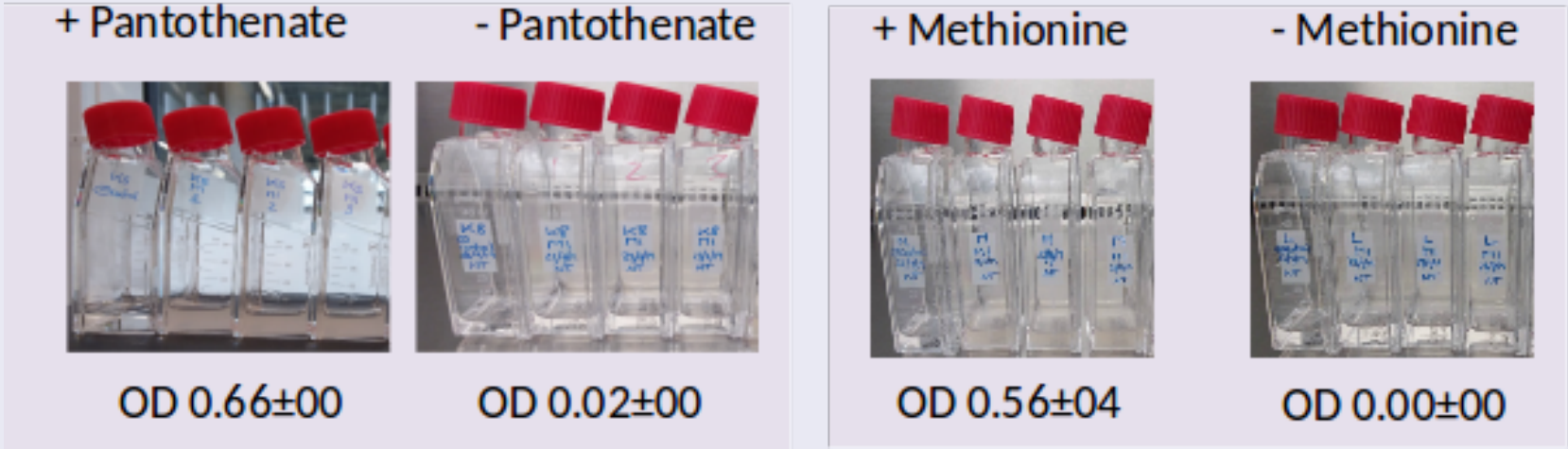

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Amino acids/Pyruvate preference

- 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/Glutamate	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. 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

Acknowledgement

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Emily Stoakes
Andrew Grant

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Fauzy Nasher
Brendan Wren

Oxford Brookes University
Mark Poolman

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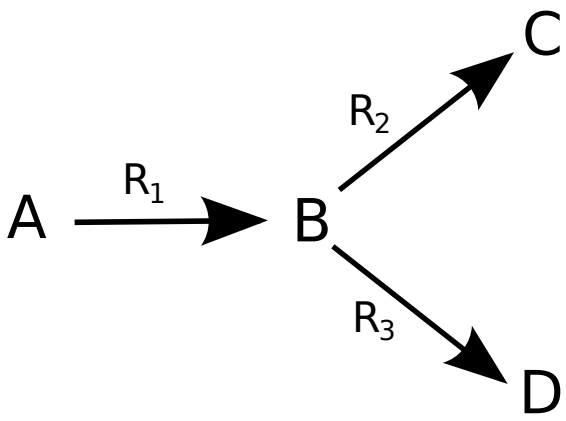
Reaction Network to Mathematical Object

Hypothetical network



Stoichiometric matrix

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

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

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

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

$$\begin{array}{lll} \text{minimise}|\text{maximise} & : & \mathrm{v}_{targs} \quad \text{objective function} \\ \text{constraints} & & \begin{cases} \mathrm{N} \cdot \mathrm{v} = 0 & \text{steady-state constraint} \\ max_i \geq v_i \geq min_i & \text{media constraint} \\ v_{i..j} \geq t_{i..j} & \text{biomass constraint} \\ \mathrm{v}_{\mathrm{ATPase}} = ATPase & \text{cell maintenance cost} \end{cases} \end{array}$$