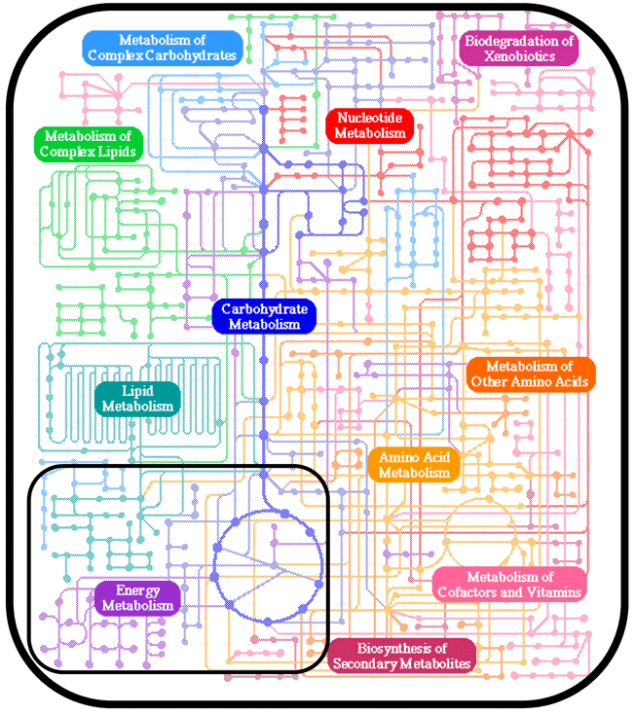
‘Modeling the metabolism of Non-Aureus Staphylococci (NAS) capable of growing in prosthetic joints’



C1NET Nottingham 2018

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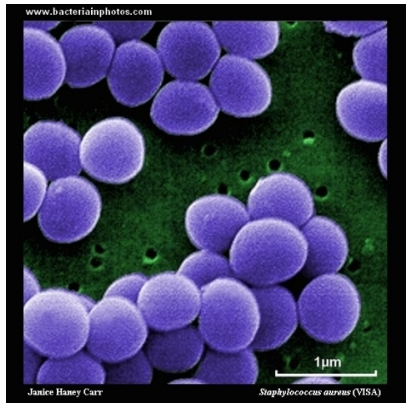
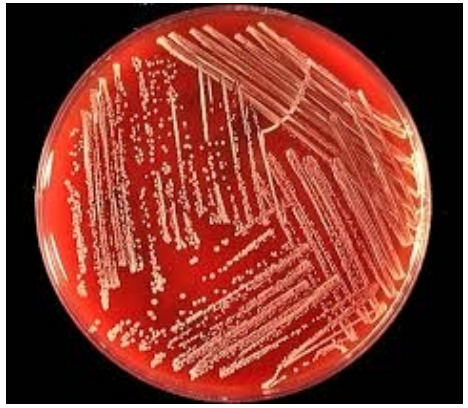
Non-Aureus Staphylococci (NAS)

Genus *Staphylococcus*
(47 species)

***S. aureus**:* a well known **highly virulent** opportunistic pathogen involved in skin and respiratory infections, food poisoning and bacteraemia





NAS…

•… most of them are **skin commensals** and **opportunistic pathogens** under certain circumstances: **far less virulent** than *S. aureus*

Staphylococci are the major cause of Prosthetic Joint Infection (PJI) (50-60%):

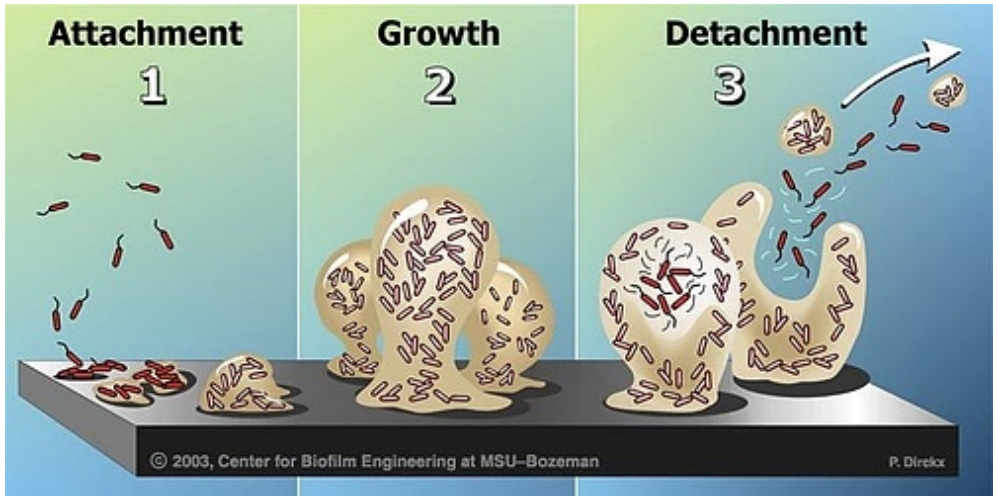
S. aureus (≃ 25-30 %) : is well understood and PJI by *S. aureus* is easy to diagnose.

NAS (≃ 25-30 %) : not well understood and diagnosis can be difficult.

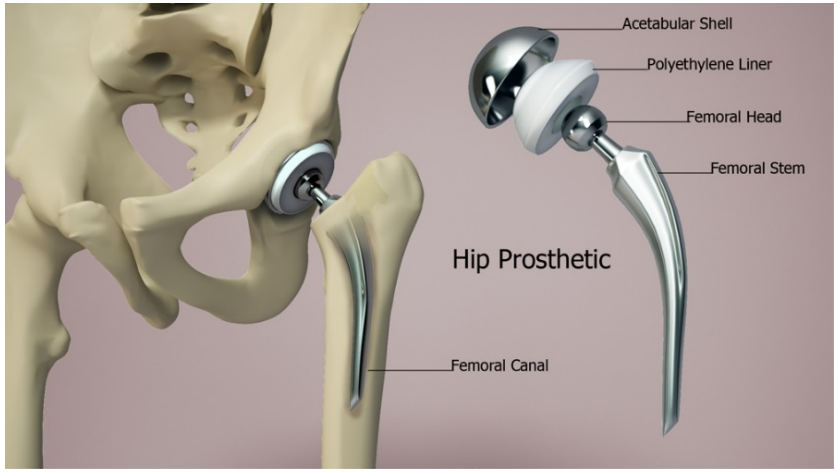
Becker, K., Heilmann, C. & Peters, G. Coagulase-negative staphylococci. *Clin Microbiol Rev* **27**, 870-926 (2014).

Why are biofilms important in prosthetic joint infection?

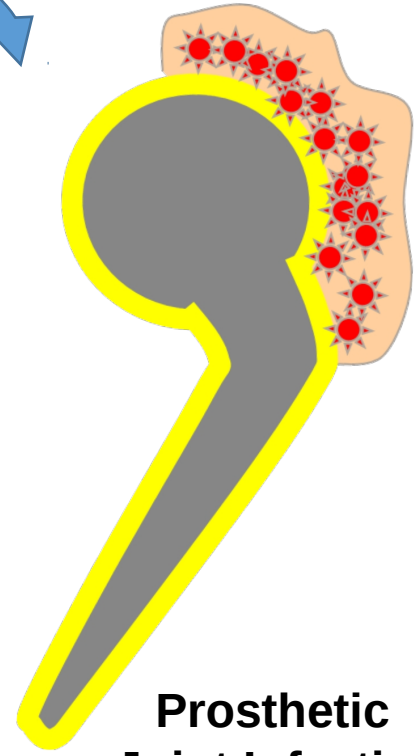
The ability to form **biofilms** is the **main pathogenic feature** of **NAS**!



Biofilms are multicellular structures where bacterial cells are encased in an extracellular matrix composed of exopolysaccharides, proteins, teichoic acids and eDNA



- Very difficult to remove
- Reduced susceptibility to antibiotics
- Protected from the host immune system



Prosthetic Joint Infection

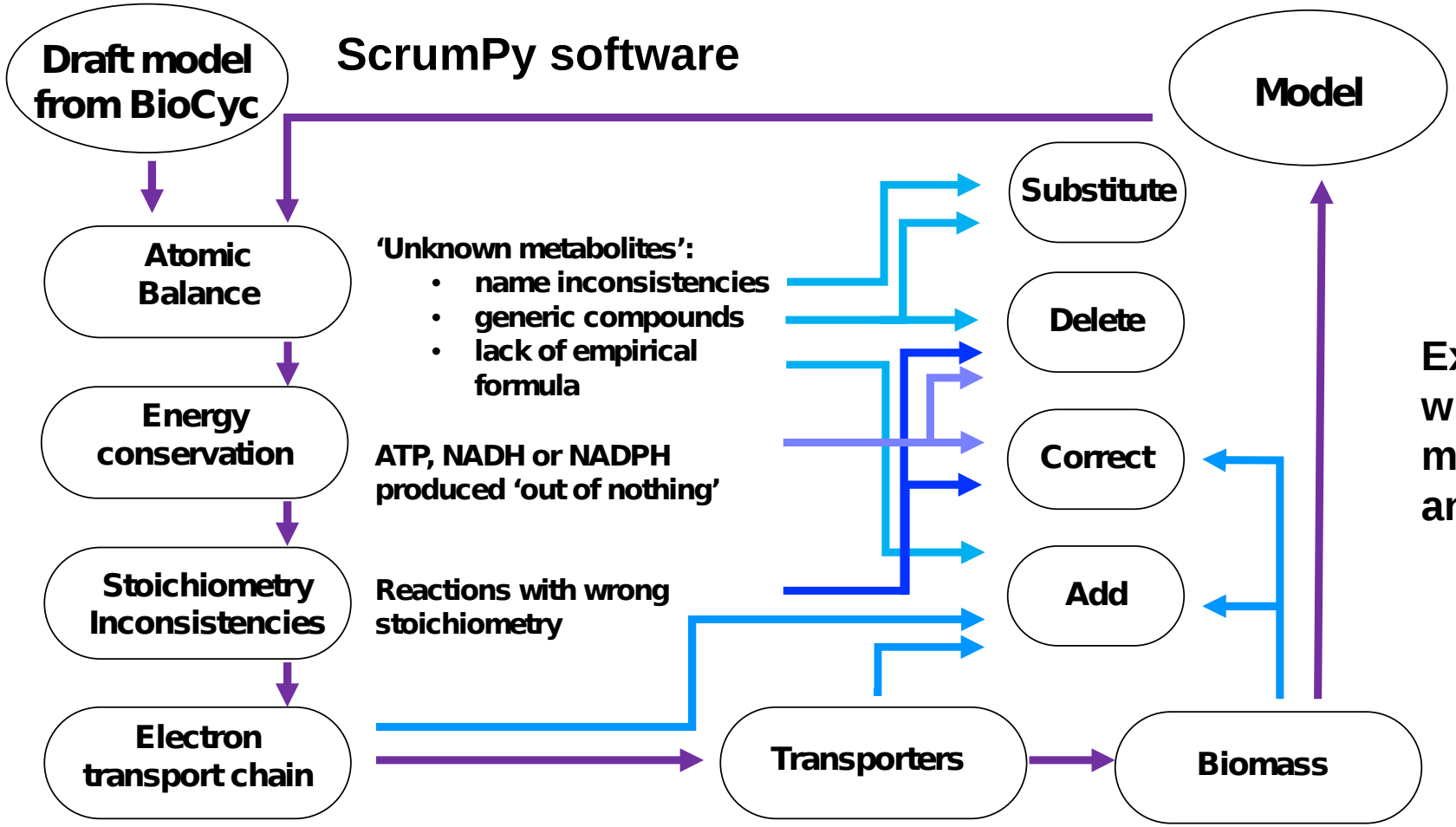
AGRESSIVE AND EXPENSIVE TREATMENT

- We need to improve diagnosis !
- We need to improve treatment !

Tande, A.J. & Patel, R. Prosthetic joint infection. *Clinical microbiology reviews* **27**, 302-345 (2014).

Constructing a structural GSM of NAS

…involves several cycles of intensive manual curation work!



Experimental data will be used for model improvement and validation.

Diagram adapted from previous work by Cell Systems Modelling Group, Oxford Brookes

Mathematical techniques used in model construction and analysis with ScrumPy

GSMs are **structural models**: study a system in the **steady state** (dynamic equilibrium)

Metabolic network represented as a **stoichiometry matrix.**

NULL SPACE ANALYSIS:

$N * v = 0$

N = stoichiometry matrix
v = velocities (unknown)

- no changes on net metabolites concentrations with time
- system of balance equations for metabolites described
- **establishes relationships between reaction fluxes**

Poolman, M.G., Sebu, C., Pidcock, M.K. & Fell, D.A. Modular decomposition of metabolic systems via null-space analysis. *Journal of theoretical biology* **249**, 691-705 (2007).
Schuster, S., Dandekar, T. & Fell, D.A. Detection of elementary flux modes in biochemical networks: a promising tool for pathway analysis and metabolic engineering. *Trends in biotechnology* **17**, 53-60 (1999).
Fell, D.A. & Small, J.R. Fat synthesis in adipose tissue. An examination of stoichiometric constraints. *Biochemical Journal* **238**, 781-786 (1986).

TECHNIQUES DERIVED FROM NULL SPACE ANALYSIS:

1) Dead reactions

- reactions which **can not carry flux** at steady-state.

2) Reaction subsets:

- reaction sets that must **carry flux in a fixed ratio** in any steady-state flux distribution

3) Elementary modes analysis (EMA):

- finds **all feasible balanced routes** through the system.

4) Flux Balance Analysis (FBA):

- finds feasible solutions **subjected to flux constraints** and an **objective function** given by the user.

Poolman, M.G., Sebu, C., Pidcock, M.K. & Fell, D.A. Modular decomposition of metabolic systems via null-space analysis. *Journal of theoretical biology* **249**, 691-705 (2007).
Schuster, S., Dandekar, T. & Fell, D.A. Detection of elementary flux modes in biochemical networks: a promising tool for pathway analysis and metabolic engineering. *Trends in biotechnology* **17**, 53-60 (1999).
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Metabolism of staphylococci

- Gram-positive
- Facultative anaerobes: aerobic or anaerobic respiration
  - can use O_2 or NO_3 as final electron acceptors
- Fermentative behaviour in absence of O_2 or NO_3:
  - lactate is the main fermentation product.
  - others: acetate, formate and CO_2 (traces).
- Produce biofilms:
  - cell live in a microaerobic environment (with oxygen gradient through biofilm).
  - cells present mainly a fermentative behaviour.
  - acetoin and butanediol: believed to be produced to prevent excessive acidification.

Heinemann, M., Kummel, A., Ruinatscha, R. & Panke, S. In silico genome-scale reconstruction and validation of the Staphylococcus aureus metabolic network. *Biotechnol Bioeng* **92**, 850-864 (2005).
Zhu, Y. et al. *Staphylococcus aureus* biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. *Infect Immun* **75**, 4219-4226 (2007).
Anaerobic Glucose and Serine Metabolism in *Staphylococcus epidermidis* 1980.

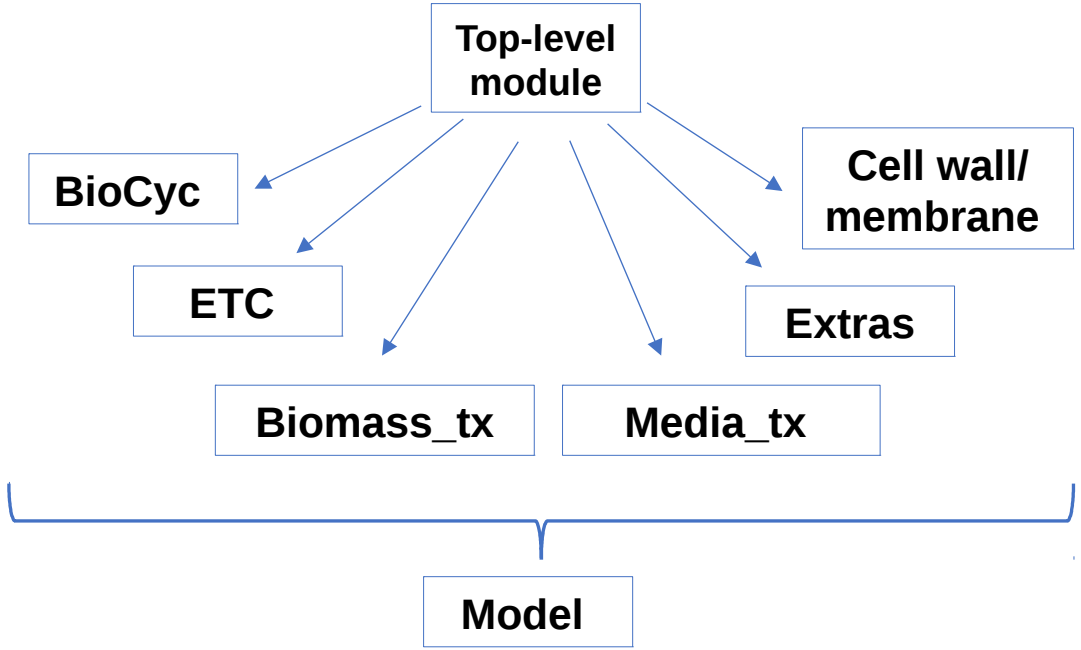
Genome Scale Model

Model description

- GSM constructed from BioCyc database for a NAS strain of interest.
- Model defined as a set of modules (961 reactions, 853 metabolites) =

Top-level module that imports the others:

A) Reactions directly imported from BioCyc (833)
B) Full electron-transport chain (5)
C) Transporters for biomass components (46)
D) Transporters for glucose minimal media and metabolic by-products (29)
E) Extra reactions for completion of cell membrane/wall synthesis (34)
F) Other additional reactions (15)



- Independent modules defined for production of cell membrane/wall components (glycolipids, phospholipids, apolar lipids, wall-teichoic acid and peptidoglycan), some of which will be important for biofilm formation.

GSM improvement

Reaction subsets = 408
- inconsistent = 1

(could be safely removed)

No net stoichiometric inconsistencies = 0

GSM PROGRESS TO DATE	BEFORE CURATION	AFTER CURATION
Reaction number	1348	961
Metabolites	1411	853
Dead reactions	767	352
Orphan metabolites	624	361
Metabolites with undefined empirical formula	564	43
Unbalanced reactions	579	0
Transporters	Automatically generated (removed)	Added manually
Atomic balance for C, N, S, P, O, H	No	Yes
Biomass composition	None	Added manually (*S. aureus*)
Energetic inconsistencies	Yes	None
Biomass production	23 out of 46 components	3 amino acids (auxotrophies)

The electron transport chain

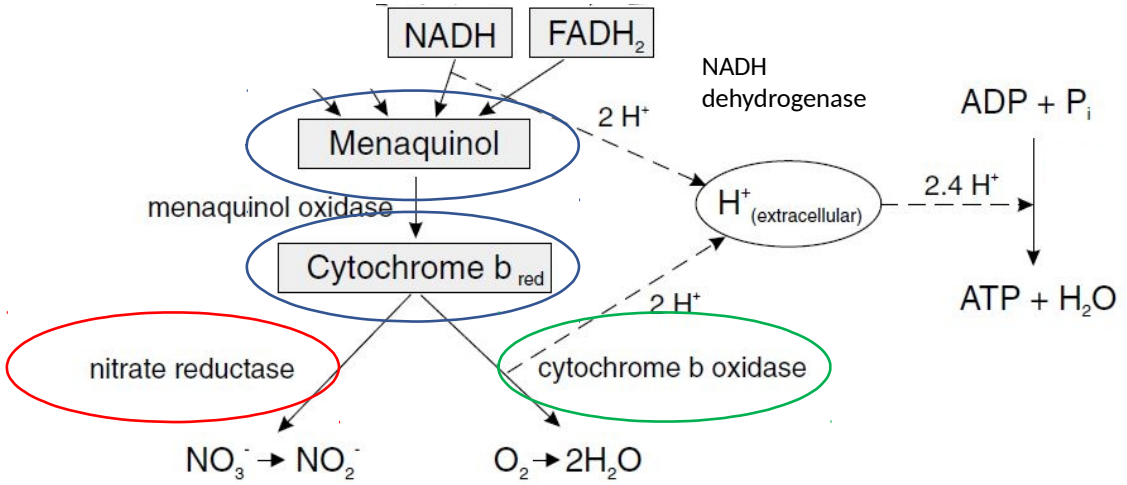
- **Fully-functional** aerobic and anaerobic **respiratory chain** ✓
- **PMF generated** by =
  - NADH menaquinone-dependant dehydrogenases
  - Cytochrome-b oxidase
- Final electron acceptors : **O_2 or NO_3**
- ETC working as described in the model by Heinemann (iMH551) although **PROTON stoichiometry** for PMF generation and ATP synthesis as been modified **as per MetaCyc**.

The respiratory chain:

Aerobic respiration

Anaerobic respiration

- both same apparatus: electron carriers
- different e- final acceptors (O_2 or NO_3^-)



Solid lines: enzymatic reaction
Broken lines: proton translocation processes

Heinemann, M., Kummel, A., Ruinatscha, R. & Panke, S. In silico genome-scale reconstruction and validation of the Staphylococcus aureus metabolic network. *Biotechnol Bioeng* **92**, 850-864 (2005).

Model analysis (FBA, EMA)

Electron transport chain module: elementary modes

ElMo_0: 2/9 x_O2 + 4/9 x_NADH + 4/9 x_PROTON -> 4/9 x_NAD + 1 x_AWork + 4/9 x_WATER

ElMo_1: 4/7 x_NADH + 4/7 x_NITRATE + 4/7 x_PROTON -> 4/7 x_NAD + 4/7 x_NITRITE + 1 x_AWork + 4/7 x_WATER

ElMo_2: 8/15 x_NADH + 2/15 x_NITRATE + 4/5 x_PROTON -> 8/15 x_NAD + 1 x_AWork + 2/15 x_NH4 + 2/5 x_WATER

ATP/NADH ratios

ElMo_0: 2.25

ElMo_1: 1.75

ElMo_2: 1.87

- Using O2 as final electron acceptor is more efficient
- Reducing NO_3 to ammonia is more efficient than reducing it to NO_2 and excreting it.

ATP production

- Without considering biomass production
- GLC-based minimal media (glucose + core set substrates)
- Fixed flux through ATPase =1

ELECTRON ACCEPTOR	MIN TOTAL FLUX (A)	MIN GLC USAGE (B)	ATP / GLC
O_2 / NO_3	*Oxidation to ACET + ETC with O_2.	Glycolysis + TCA cycle + ETC with O_2	A) 11 B) 26
O_2	*Oxidation to ACET + ETC with O_2.	Glycolysis + TCA cycle + ETC with O_2	A) 11 B) 26
NO_3	*Oxidation to ACET and uses ETC with NO_3	Glycolysis + TCA cycle + ETC with NO_3	A) 9.5 B) 21.5
-	Ferments to ACET, ETOH and SUC Would ferment to D-LAC if ACET_tx and SUC_tx are blocked together (ATP / GLC =2).	Ferments to ACET. Would ferment to any other fermentation product before D-LAC. Would ferment to lactate if blocking all other by-products transporters except acetoin. (ATP / GLC =2).	A) 3 B) 4

*If flux through the acetate transporter is blocked, the model will carry out glycolysis.

Biomass and metabolite production

- **In silico GLC-based minimal media defined**: core set substrates (ammonium, sulphate, inorganic phosphate), glucose, and three essential amino acids (MET, GLY, ASN).

- **Investigated with and without O_2 and NO_3**

- **All components produced except for the 3 amino acids:** staphylococcal strains often present various amino acid auxotrophies which vary depending on strains, so this is not surprising.

Xavier, J.C., Patil, K.R. & Rocha, I. Integration of Biomass Formulations of Genome-Scale Metabolic Models with Experimental Data Reveals Universally Essential Cofactors in Prokaryotes. *Metabolic engineering* **39**, 200-208 (2017). Heinemann, M., Kummel, A., Ruinatscha, R. & Panke, S. In silico genome-scale reconstruction and validation of the Staphylococcus aureus metabolic network. *Biotechnol Bioeng* **92**, 850-864 (2005).
Zhu, Y. et al. *Staphylococcus aureus* biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. *Infect Immun* **75**, 4219-4226 (2007).

Biomass and metabolites production

- **Production of vitamins and cofactors tested:** pantothenate, riboflabin (produced); thiamine, niacine and biotin (not produced). Not included on biomass description. Experimental evidence for most *S. aureus* strains needing thiamine and niacine plus other vitamins (depending on strains) for growth. Niacine has been included in the minimal media since is needed for production of biomass components under anaerobic conditions (NADH, NADPH).
    - PROTOHEME and SIROHEME (prosthetic groups of cytochrome-b oxidoreductase and nitrate reductase respectively) can be produced if Fe^{2+} is added to the media. These groups are not directly involved in any reactions in the model.

- **Fermentation by-products** produced by the model: lactate, acetate, formate, ethanol, acetoin, succinate, 2-ketoglutarate, butyric acid and butanol.

Xavier, J.C., Patil, K.R. & Rocha, I. Integration of Biomass Formulations of Genome-Scale Metabolic Models with Experimental Data Reveals Universally Essential Cofactors in Prokaryotes. *Metabolic engineering* **39**, 200-208 (2017). Heinemann, M., Kummel, A., Ruinatscha, R. & Panke, S. In silico genome-scale reconstruction and validation of the Staphylococcus aureus metabolic network. *Biotechnol Bioeng* **92**, 850-864 (2005).
Zhu, Y. et al. *Staphylococcus aureus* biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. *Infect Immun* **75**, 4219-4226 (2007).

Model validation

Model prediction *vs* literature data:

The model is balanced for water and protons: have to even be considered in fermentative behaviour (good indicative of the quality of the model).

ATP/GLC = 26 through glycolysis + TCA (iMH551?)
P/O ratio =
- oxidative + substrate level phosphorylation = 2.17
- oxidative phosphorylation = 1.83 (1.3 reported for GLT Tynecka et al.,1999)

ATP/NADH = 2.25 (generally assumed 2 for *S. aureus*, Wilkinson, 1997)
H/ATP = 4 (As per MetaCyc; 2.4 Heinemann, 3.3 *E.coli* Jiang et al., 2001)

Main fermentation product =

- **acetate** and other by-products **rather than lactate** (in literature): which indicates that the strain metabolisms *in vivo* is not directed to minimize reaction fluxes or GLC usage for ATP production (the actual biological objective is different).

- **another hypothesis** to explain this discrepancy: **it is due to a thiamine auxotrophy**. FBA demonstrated that in absence of O_2 and NO_3, if the flux of reactions catalysed by enzymes dependent on thiamine pyrophosphate is constrained to 0, the model ferments all glucose to D-lactate (matching experimental results).

Somerville, G.A. et al. Correlation of acetate catabolism and growth yield in Staphylococcus aureus: implications for host-pathogen interactions. *Infection and immunity* **71**, 4724-4732 (2003).
Parisi, J.T. Coagulase-negative staphylococci and the epidemiological typing of Staphylococcus epidermidis. *Microbiological Reviews* **49**, 126-139 (1985).
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Heinemann, M., Kummel, A., Ruinatscha, R. & Panke, S. In silico genome-scale reconstruction and validation of the Staphylococcus aureus metabolic network. *Biotechnol Bioeng* **92**, 850-864 (2005).

Model validation

Vitamin auxotrophies: need for niacine to produce NADH and NADPH matches experimental data and predictions by iMH5551. The need for thiamine and biotin has been described experimentally but is not required to produce biomass by the model since they are needed to produce prosthetic groups (again, matching predictions with iMH551).

Differences with *S. aureus* model iMH551 (by Heinemann *et al* 2005): NONE amino acid auxotrophies were defined with iMH551 and an uracil auxotrophy detected to produce DNA/RNA in anaerobiosis (also described experimentally for *S. aureus*) was not seen with my model.

'The glucose effect': downregulation of TCA activity when growing in aerobiosis in excess of GLC**.** This was reproduced by constraining flux through the **aconitate hydratase reaction**. Acetate excretion detected experimentally matches the behavior of the model predicted with FBA (if objective function= min total flux).

Acetoin utilization pathway: (with FBA solution for: Objective= min flux ; ATPase flux =1 ; with the *in silico* defined minimal media and allowing uptake of all possible fermentation products) = pathway described in biofilm metabolism in NAS.

ElMo_0: 1 x_O2 + 1 x_ACETOIN -> 2 x_ACET + 2 x_PROTON + 6 x_AWork

Somerville, G.A. et al. Correlation of acetate catabolism and growth yield in Staphylococcus aureus: implications for host-pathogen interactions. *Infection and immunity* **71**, 4724-4732 (2003).
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Heinemann, M., Kummel, A., Ruinatscha, R. & Panke, S. In silico genome-scale reconstruction and validation of the Staphylococcus aureus metabolic network. *Biotechnol Bioeng* **92**, 850-864 (2005).

What to do next

Model analysis:

- Define an approximate **composition for NAS biofilms** (from literature).
- **Flux Balance Analysis:**
  - Define if there is a **change in the reactions flux** associated with **biofilm production.**
  - **ATPScan**: define the **‘catabolic core’** of the model under different conditions (+/- O_2 and NO_3 and in stages to allow for sequential uptake of metabolic by-products)
  - **Gene-knockouts Analysis** (define essential single genes and double gene knockouts for biofilm formation and ATP production).
- **Elementary Modes Analysis :** define elementary modes **for production of biofilm components**.

What to do next

Experimental (model validation):

- Obtain accurate **biomass composition data** for **planktonic cells vs biofilms** in order to improve accuracy of model predictions.
- **Compare growth in a minimal media** for staphylococcus previously defined in the literature **with the minimal media defined in-silico** with my model.
- Perform **Biolog experiments**: study nutrient utilization for growth and auxotrophies and compare them with model predictions.
- Use of a **library of transposon mutants** for validation of essential genes for growth defined by FBA.

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University of East Anglia

Thanks for listening!

University of East Anglia