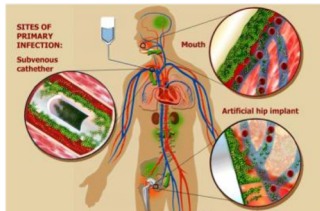


The acquisition of additional feedback loops optimizes and speeds up the response of Quorum Sensing

Marco Fondi
IBCWG August 25, 2022

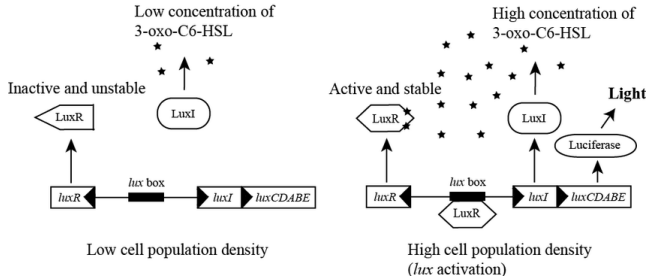
Quorum sensing

Bacterial QS is a cell-to-cell communication system in which specific signals are activated to coordinate, for example, pathogenic behaviors and help bacteria collectively respond to perturbations.



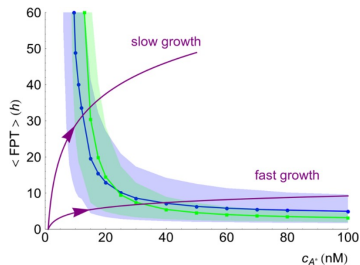
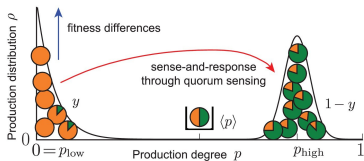
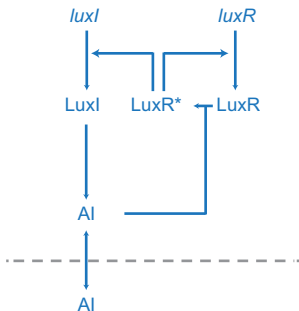
It **regulates** bacteria biofilm formation and virulence in general.

The *V. fischeri* LuxIR system

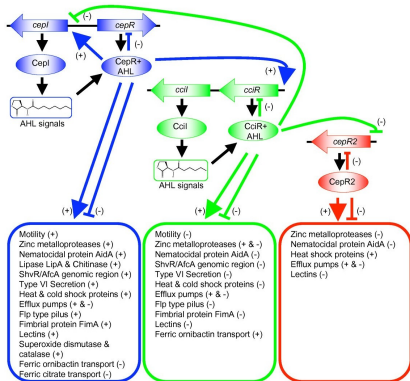


LuxR is an autoinducer (AI)-dependent positive regulator of the lux operon, and LuxI produces the AI molecule, 3-oxo-hexanoyl-L-homoserine lactone

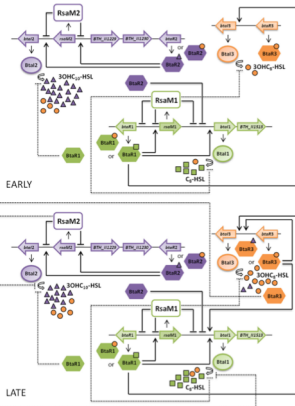
Bistability and robustness in QS regulation



Multiple QS regulation system and their cross-talk in *Burkholderia*

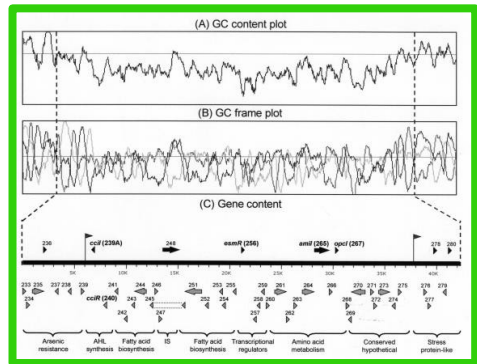
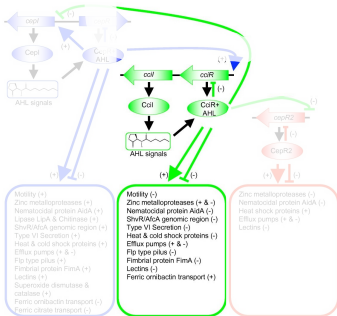


O'Grady et al. 2009, BMC Genomics



Le Guillouzer et al. 2018

The CciIR regulators are located on the *cci* genomic island



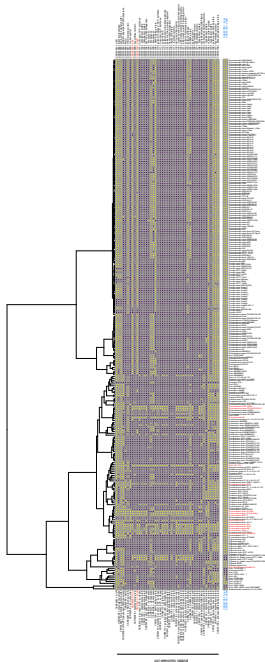
Baldwin et al. 2004

The *Burkholderia cepacia* epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in *Burkholderia cenocepacia*

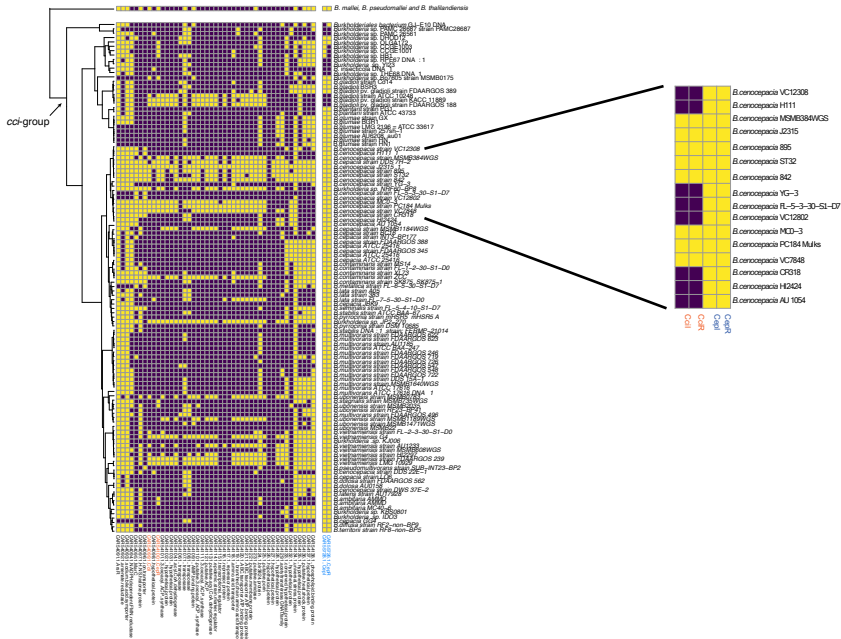
Aims and motivation

The inclusion of a regulatory system (CciIR) inside a genomic island (*cci*) represents an extraordinary example of the plasticity of the cellular transcriptional network and an exceptional occasion to study the effect of alternative regulatory architectures on the expression of the resulting phenotypic trait.

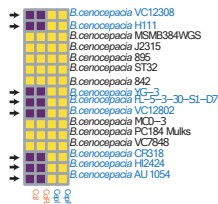
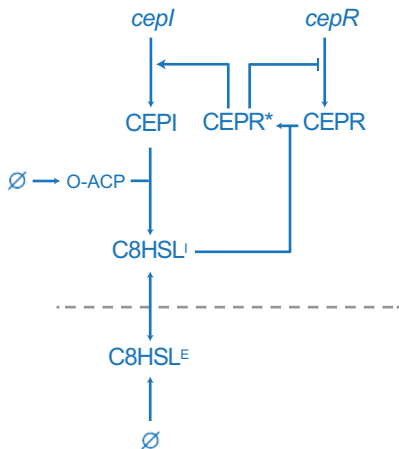
- Which is the actual distrubution of the *cci*-encoded CciIR regulatory system in *Burkholderia*?
- Which are the evolutionary advantages provided by the acquisition of additional control over bacterial regulation of QS?



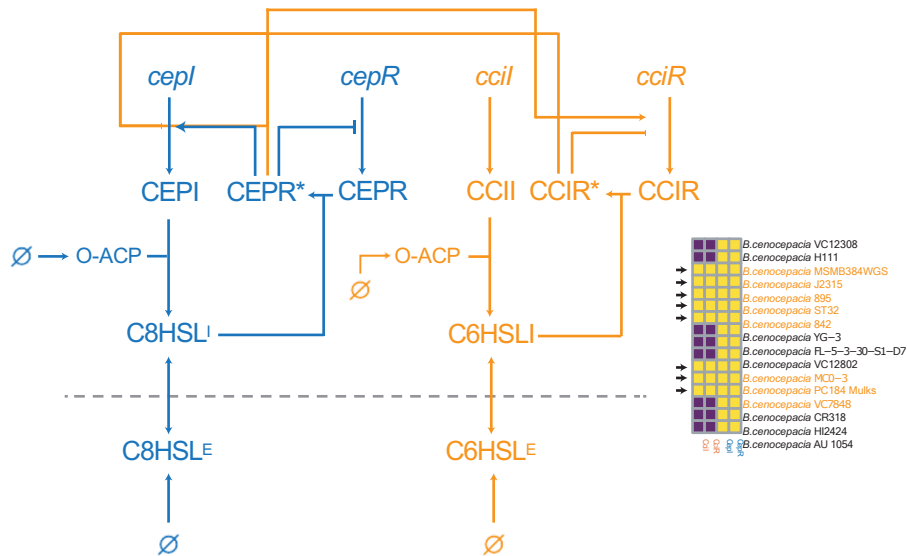
- 289 *Burkholderia* completely sequenced genomes probed using *B. cenocepacia* J2315 *cci* island genes (using Bidirectional Best Hit method)
- Rows (genomes) clustered according to shared ANI values
- In *B. mallei*, *B. pseudomallei* and *B. thalilandiensis* the complete set of *cci* genes is never found. In the other cluster (hereinafter *cci* -group the distribution is patchy and includes (22) microbes that harbour more than 50% of the reference *cci* genes and others (93) possessing less than 50% of the reference *cci* genes.



Two alternative QS regulation architectures: core



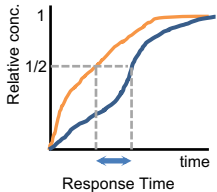
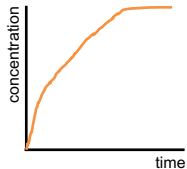
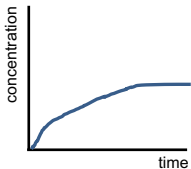
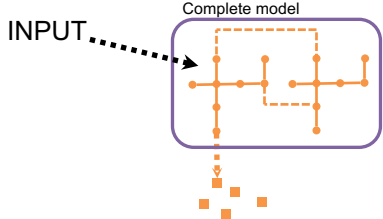
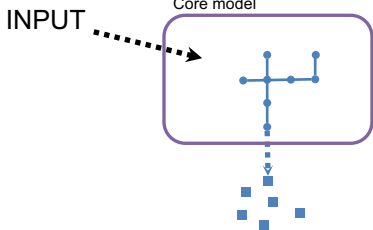
Two alternative QS regulation architectures: complete



Aims and motivation

The inclusion of a regulatory system (CciIR) inside a genomic island (*cci*) represents an extraordinary example of the plasticity of the cellular transcriptional network and an exceptional occasion to study the effect of alternative regulatory architectures on the expression of the resulting phenotypic trait.

- Which is the actual distribution of the *cci*-encoded CciIR regulatory system in *Burkholderia*?
- Which are the evolutionary advantages provided by the acquisition of additional control over bacterial regulation of QS?



A mathematical model accounting for the two regulatory schemes

$$\emptyset \xrightarrow{\alpha OACP} OACP$$

$$CEPI \xrightarrow{\delta} \emptyset$$

$$CEPR \xrightarrow{\delta} \emptyset$$

$$CEPR^* \xrightarrow{\delta} \emptyset$$

$$OACP \xrightarrow{\delta} \emptyset$$

$$C_s I \xrightarrow{\delta} \emptyset$$

$$CEPR + C_s I \xleftarrow[k_{off,CEPR^*}]{k_{on,CEPR^*}} CEPR^*$$

$$OACP + CEPI \xrightarrow[k_{m,CEPI+OACP}]{V_{max,CEPI} \cdot OACP} C_s I + CEPI$$

$$\emptyset \xrightarrow[\frac{\beta_2}{1 + \left(\frac{CCIR^*}{k_2}\right)^m + \frac{\beta_1 \cdot (CEPR^*)^w}{k_1 + (CEPR^*)^w}}]{\beta_2} CEPI$$

$$\emptyset \xrightarrow[\frac{\beta_1}{1 + \left(\frac{CEPR^*}{k_3}\right)^n}]{\beta_1} CEPR$$

$$\emptyset \xrightarrow{\beta_2} CCII$$

$$CCIR \xrightarrow{\delta} \emptyset$$

$$CCII \xrightarrow{\delta} \emptyset$$

$$CCIR^* \xrightarrow{\delta} \emptyset$$

$$C_o I \xrightarrow{\delta} \emptyset$$

$$CCIR + C_o I \xleftarrow[k_{off,CCIR^*}]{k_{on,CCIR^*}} CCIR^*$$

$$OACP + CCII \xrightarrow[\frac{V_{max,CCII} \cdot OACP}{k_m,CCII+OACP}]{V_{max,CCII} \cdot OACP} C_o I + CCII$$

$$\emptyset \xrightarrow[\frac{\beta_2}{1 + \left(\frac{CCIR^*}{k_4}\right)^m + \frac{\beta_2 \cdot CEPR^{*w}}{k_5 + CEPR^{*w}}]{\beta_2} CCIR$$

$$C_s I \xleftarrow{\alpha C_s} C_s E$$

$$C_o I \xleftarrow{\alpha C_o} C_o E$$

$$C_s E \xrightarrow{\delta} \emptyset$$

$$C_o E \xrightarrow{\delta} \emptyset$$

$$\emptyset \xrightarrow{\gamma C_s} C_s E$$

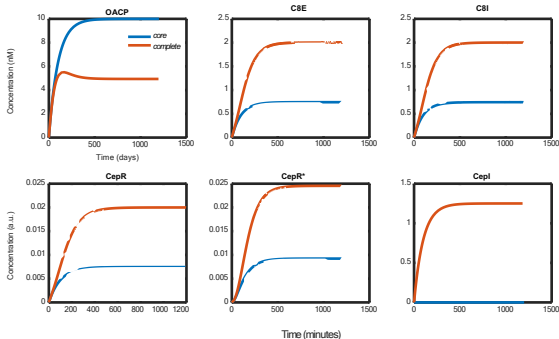
$$\emptyset \xrightarrow{\gamma C_o} C_o E$$

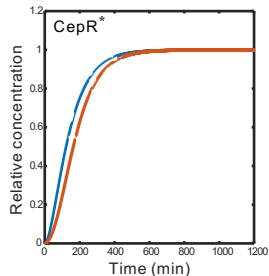
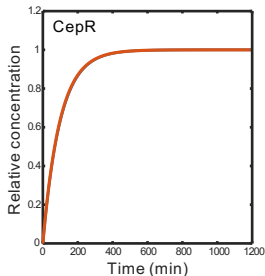
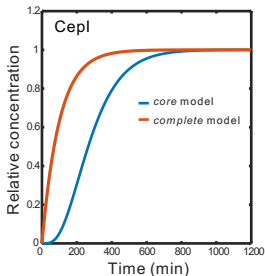
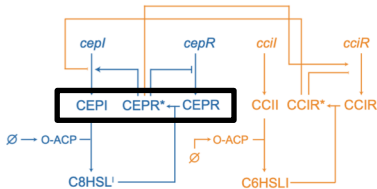
All models are wrong
but some are useful
(George E. P. Box)



Simulating the system to its steady state

We simulated the behaviour of the system for 1200 minutes upon its activation (all initial concentrations set to 0) and evaluated the dynamics of the main species included in the model

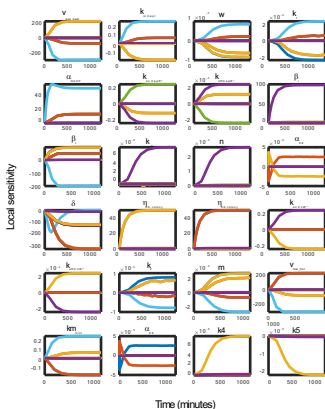




The two circuits differ in their response time (RT)

The RTs are **69** and **264** minutes in the case of the complete and the core models, respectively

We can now use the model to address an important question: How does QS respond to external parameters variation?

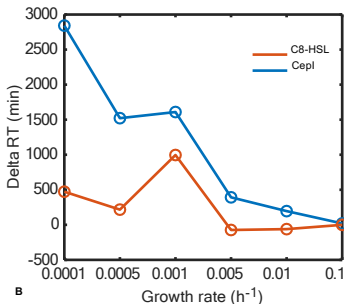
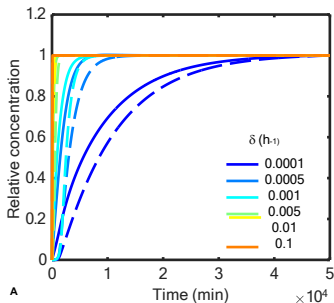


- Local sensitivity analysis:

$$S_{ij} = \frac{dx_i(t_n)}{dp_j} \quad (12)$$

- the model is robust to variations in the values of most parameters as local sensitivity is mostly maintained in the range of $1 * 10^{-2} / 1 * 10^{-4}$. Exceptions are represented by $V_{max,CEPI}$, α_{OACP} , β_1 , β_2 , δ , $\gamma_{C8,colony}$, $\gamma_{C6,colony}$ and $V_{max,Ccil}$ for the *complete* model

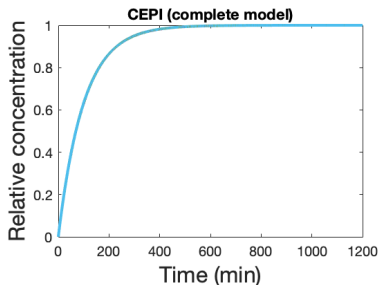
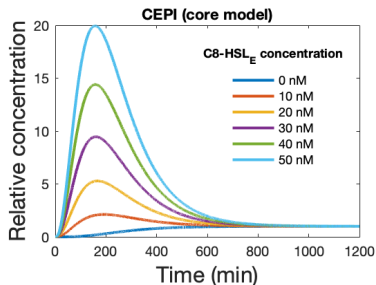
We repeated the simulations described above for different values of δ . Concerning the response time of Ceph, we found a strong influence of δ on the RT of the complete and core models.



Response time in the two configurations

The complete model provides a faster response than the core one. This difference is higher at low growth rates

Does the AI concentration influence the response?

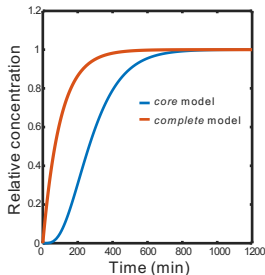


- In the core model, with higher concentrations of AI, CepI is predicted to reach levels that are very far from the optimal, steady state concentration

Optimization in response to AI concentration (1)

The complete model is optimized in response to external AI concentration

Conclusions



The *complete* model has a shorter RT

The efficacy of the *complete* model over the *core* one is maximized in two conditions: **i) low growth rates and ii) high cell density**

Low growth rates are typically encountered by free living bacteria exposed to nutritional stress during the establishment of **host infection** (restricted nutritional conditions with low cell density). Additionally, combined low growth rates and high cell density are encountered by bacteria that live in **biofilms**.

Working hypothesis: is the complete scheme helpful during infections?

We speculate that the additional feedback loops provided by the CciIR regulation system may provide an evolutionary advantage to the harbouring strains during the onset and maintenance of **host infections**.

Acknowledgements

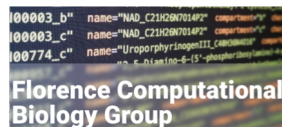
University of Florence

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Read more on BioRxiv (same title)...

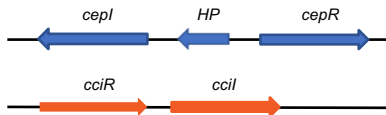
Open positions
in the lab!



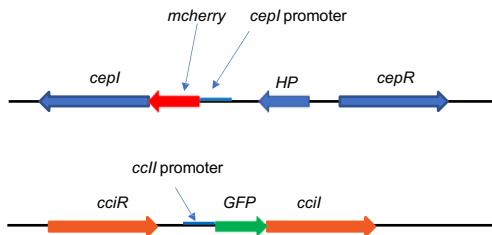
MINISTERO DELL' ISTRUZIONE, DELL'UNIVERSITÀ E DELLA RICERCA

Experimental validation

1. Deletion of *cepI*, *cepR*, *cciI* and *cciR* and of both genes



2. Insertion of reporter genes downstream of the promoters of the two inducers



The regulation of QS and QS-related processes has been almost exclusively interpreted as (cell) density-dependent feature. However, mounting evidence suggests that growth rate, in addition to cellular concentration, plays a major role in triggering this complex phenotypic response.

- *Vibrio fischeri*
- *Pseudomonas aeruginosa*
- *B. glumae*
- *B. cenocepacia*

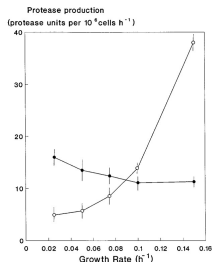


FIG. 2. Effect of specific growth rate upon the production of protease by *B. cenocepacia* in an iron-limited, chemically defined simple salts medium. Protease content was expressed as productivity under oxygen-replete (○) or oxygen-depleted (●) conditions.

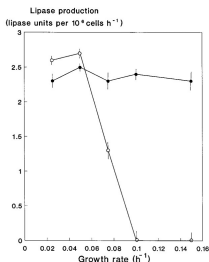
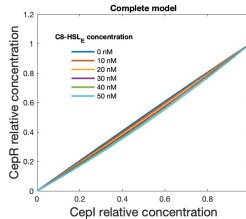
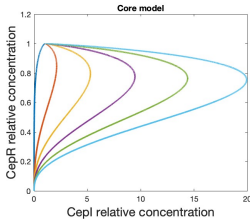
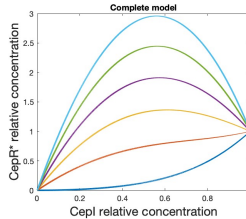
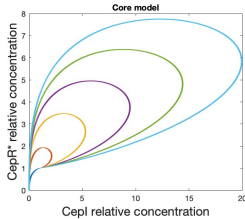


FIG. 3. Effect of specific growth rate upon the production of lipase by *B. cenocepacia* in an iron-limited, chemically defined simple salts medium. Lipase content was expressed as productivity under oxygen-replete (○) or oxygen-depleted (●) conditions.

CepI vs. CepR and CepI vs. CepR* in the phase plane

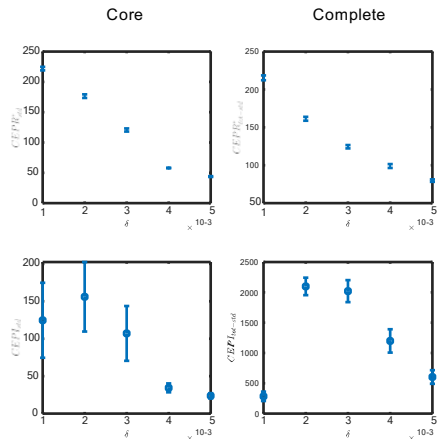


Optimization in response to AI concentration (2)

The complete model has a "simpler" phase plane

Stochastic simulations

Heterogeneity is ubiquitous across all domains of life. In microbes phenotypic emerges as a result of multiple factors such as fluctuations in the microenvironment, stochasticity in gene expression.



Smaller fluctuations in the *core* configuration. Better response to fluctuating environmental conditions?

Negative Autoregulation Speeds the Response Times of Transcription Networks

Nitzan Rosenfeld¹, Michael B. Elowitz² and Uri Alon^{1*}

