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FIGHTING AGAINST THE GENETIC QUORUM SENSING OF *LEGIONELLA PNEUMOPHILA*

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RESEARCH PROJECT 2017 – 2018

WHY *L. pneumophila?* WHY QUORUM SENSING?

***L. pneumophila*:**

- Causes Legionnaire's disease, a sickness very difficult to treat due to antibiotic resistances in *L. pneumophila*[1].
- Has a biphasic life cycle: a replicative phase and a virulence phase (Fig. 1). The switch between both phases is controlled by a Quorum Sensing System[2].

Quorum sensing (QS):

- Means bacterial language.
- Coordinates the gene expression of a bacterial community.

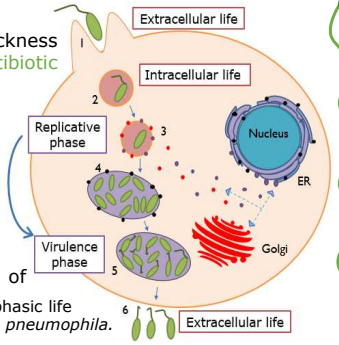


Fig. 1; Biphasic life cycle of *L. pneumophila*.

L. pneumophila QUORUM SENSING (Lqs)

The Lqs system regulates gene expression with 4 elements[2] (Fig. 2):

1. LqsA: Autoinducer synthetase, produces LAI-1
2. LqsS /LqsT: Membrane receptors, detect LAI-1
3. LAI-1: Autoinducer, turns on the Lqs system
4. LqsR: Response regulator, activates or reprimes gene transcription

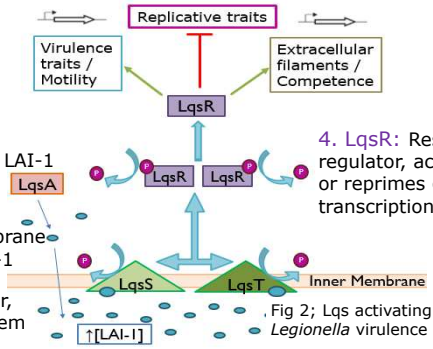


Fig 2; Lqs activating the *Legionella* virulence

HYPOTHESIS

1. Blocking the activation of the Lqs system we can block the *L. pneumophila* virulence capacity.
2. *Legionella pneumophila* and *Vibrio cholerae* have two QS systems with the same evolutionary origin[3].

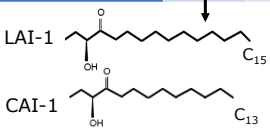
COMPARING QS SYSTEMS IN *L. pneumophila* AND *V. cholerae*

3. Quorum Sensing Inhibitor compounds (QSI) have been identified for being able to block the QS in *Vibrio*[4].
4. According with the evolutionary homology, could we use the *Vibrio* QSI mechanism to block the Lqs system?

ANALOGOUS QS SYSTEMS: Lqs – Cqs

Lqs (*L. pneumophila*)	LAI-1	LqsA	LqsS/T	LqsR
Cqs (*V. choleare*)	CAI-1	CqsA	CqsS	CqsR

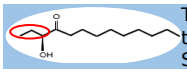
Fig 3; Elements of the Lqs and Cqs systems, both with equal elements and roles.



The three membrane receptors have conserved all the essential aminoamides, and its running its basically the same.

THE QS INHIBITION IN *VIBRIO*

Common CAI-1 binds to CqsS and activates an intracellular signalling cascade.



The QSI is created inducing changes between the C1 and C2 of the autoinducer CAI-1. So, the QSI is a CAI-1's antagonist.

QSIs are competing with CAI-1	The QSIs bind to the membrane receptor, but they do not activate the following signalling cascade. The QSIs blocks the expression of virulence genes.

THE PROJECT

OBJECTIVE

To find a *L. pneumophila*'s QSI: a single or a group of molecules which could inhibit the Lqs system, acting as competitors of LAI-1, arresting the bacterial virulence capacity.

PROPOSED METHOD TO FIND A QSI (Fig. 4)

1. Creating the new *L. pneumophila str.* MAIL001. With *lqsA* interrupted by a *gfp* insertion.
2. Preparing the library of possible QSIs molecules.
3. Exploring the interaction between each molecule and LqsS/T.
   Using strain MAIL001.
4. Evaluating the toxicology of each QSI molecule.
5. Studying *L. pneumophila*-eukaryote interaction with QSI
6. Assaying he effect of the QSI on transcription activation.
   - RNA-seq → general transcriptional analysis.
   - qRT-PCR → deeper understanding.

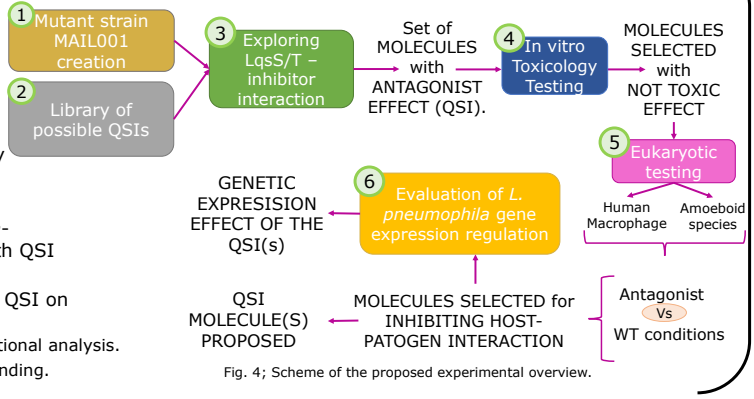
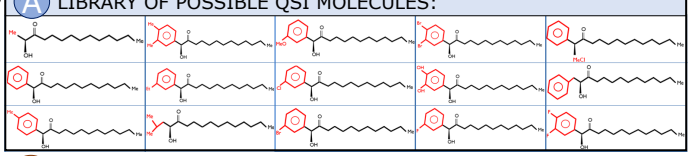


Fig. 4; Scheme of the proposed experimental overview.

EXPECTED RESULTS

A LIBRARY OF POSSIBLE QSI MOLECULES:



B MUTANT STR. *L. pneumophila* MAIL001:

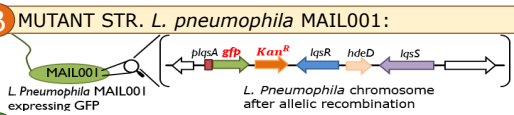


Fig. 5; MAIL001 chromosome structure

C EFFECTIVE QSI(s):

- LAI-1's competitor
- NOT toxic or mutagenic
- Inhibiting the pathogen-host manipulation
- Blocking the virulence gene expression

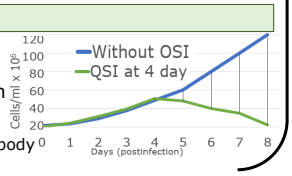


Fig. 6; Predicted *L. pneumophila cell* # in human body

PROJECT STRENGTHS

LEGIONNAIRES' DISEASE a severe health problem all over the word

EVOLUTION the hypothesis is based on the Lqs evolutionary history

FOCUS ON LQS SYSTEM AND ITS GENETIC BASE It is the virulence base of *L. pneumophila* and other bacteria

QSIs great antibiotic alternative[5]

RESISTANCES TO QSI they have not a classical selective advantage

USEFUL APPROACH the project can be applied in many other bacteria and QS systems

1. Newton H.J., *et al.* (2010). "Molecular pathogenesis of infections caused by *L. pneumophila*". Clin. Microbiol.
2. Personnic, et al. (2018). "Legionella quorum sensing and its role in pathogen-host interactions". Curr Opin Micro.
3. Tiaden A, Hilbi H. (2012). "α-Hydroxyketone Synthesis and Sensing by Legionella and Vibrio". Sensors (Basel). .
4. Bolitho ME, et al. (2011) "Small molecule probes of the receptor binding site in the V. cholerae CAI-1 QS circuit". Bioorganic Med. Chem.
5. Tom Defoirdt. (2018). "Quorum-Sensing Systems as Targets for Antivirulence Therapy". Trends in Microbiol.