What is quorum sensing? How Does it work?

- **Quorum sensing**: form of density dependent cell to cell communication among bacteria (Zhao et. al, 2020)

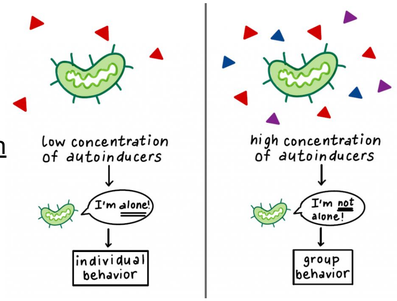
High density of bacteria

↓

Chemical secretion and signaling, i.e. communication

↓

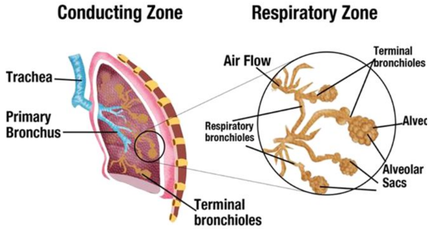
Regulatory Effects

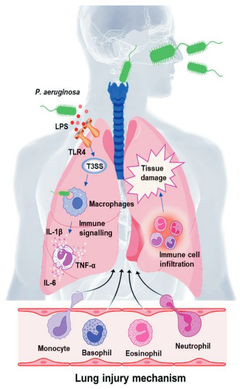


What is Biofilm

- Microorganisms attach to surfaces and produce extracellular polysaccharides that facilitate attachment and matrix formation; this results in the formation of a Biofilm
- Biofilm colony formations provide protection and a medium for intracellular signals
- Biofilm-associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents (Donlan, 2001)

Respiratory System and Microbiome



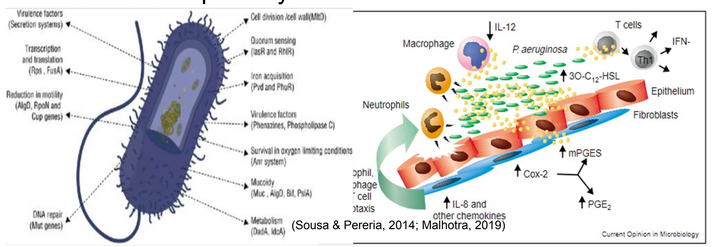


- In disease, host inflammation and immune responses, anaerobic and other conditions can benefit bacterial colonization and domination.
- Microbiome imbalance, shift towards and domination by Gammaproteobacteria such as P aeruginosa, which can lead to exacerbations of chronic lung diseases such as bronchiectasis.

(Lin, 2016; Huffnagle, 2017; Faure 2018)

Bacteria and How They Become Infectious

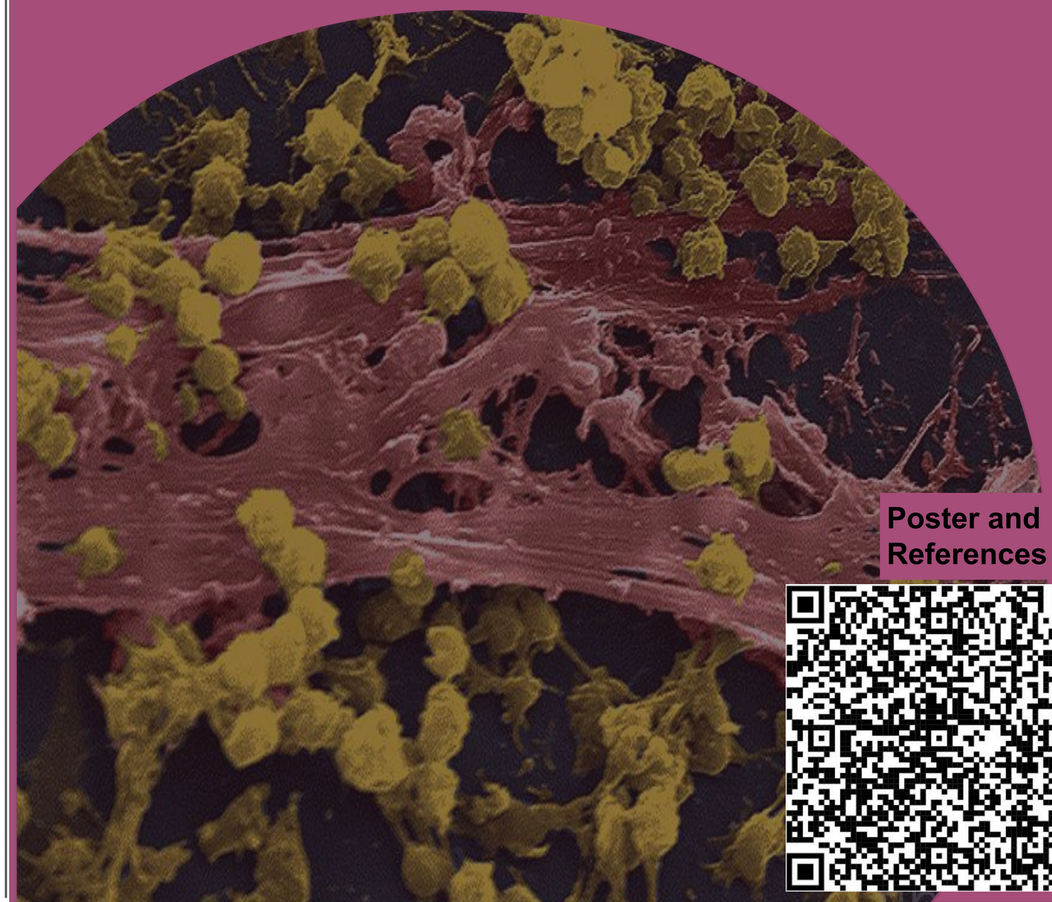
- Difficult to stop the spread
  - 6000 genes, many regulatory sequences: virulence, chemotaxis, metabolic pathways
  - Able to adapt many different environments



(Sousa & Pereria, 2014; Malhotra, 2019)

Bacteria in chronic lung disease communicates with each other to **remove** competition and **exploit** the immune system

Group 10: Bride Edo, Jacob Felix, Ziyan Kapadia, Alvin Qi, Aaron Yu, Igor Zakhidov



Poster and References

Regulatory Proteins and Gene Expression

QS contributes to the fitness of an organism, allowing the coordination of gene expression beneficial to the bacterial colony.

- Change in oprC gene after QS signals, does not affect bacteria growth but modifies inflammation response.
- The LasR regulator protein is important in coordinating gene expression in response to QS-signaling molecules in P.aeruginosa. (Joseph & Helen, 2014; Gao et al, 2020)

Quorum Sensing and Virulence

- PA is protected against the immune system
  - PA can degrade white blood cells, resists antibiotics
  - PA triggers strong inflammatory response
    - Causes damage to lung tissue
    - Body elicits more inflammatory responses caused by damages

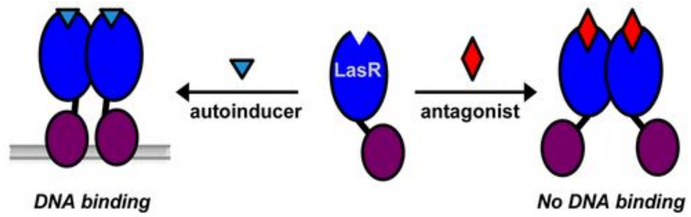
(Lin & Kazmierczak, 2017; Alhede, 2009)

- PA adapts to the host
  - Heat shock proteins upregulated
  - Alternative energy synthesis pathways in low oxygen conditions in biofilm
  - Reduces virulence factors early in establishing colony to avoid detection
    - Reduces flagellin, proteases, and other secretions

(Faure et al, 2018; Leinweber, 2018)

- PA competes with other bacterias
  - Anti-virulence proteins against other bacterias
    - Pyoverdine: Iron scavenging molecule, expression linked to intercellular competition

(Alford et al, 2022)



Potential solution

Inhibit the binding of LasR protein with molecular antagonists that mimic its original autoinducer to prevent the transmission of the QS-signaling molecules

- Inhibiting this gene expression coordinator reduces QS level and indirectly reduces bacterial virulence and infection cause by biofilm formation.
- Only targets the DNA binding of QS proteins, it inhibits the communication in bacterial colonies to produce its virulence effects without impacting the growth of the bacteria.
- Dramatically decreases bacterial resistance level to the binding antagonist
  (Emma et al, 2017)

Alford, M. A., Mann, S., Akhoundsadegh, N., & Hancock, R. E. W. (2022). Competition between Pseudomonas aeruginosa and Staphylococcus aureus is dependent on intercellular signaling and regulated by the NtrBC two-component system. *Scientific Reports*, *12*(1), Article 1. https://doi.org/10.1038/s41598-022-12650-2

Alhede, M., Bjarnsholt, T., Jensen, P. Ø., Phipps, R. K., Moser, C., Christophersen, L., Christensen, L. D., van Gennip, M., Parsek, M., Høiby, N., Rasmussen, T. B., & Givskov, M. 2009. (n.d.). Pseudomonas aeruginosa recognizes and responds aggressively to the presence of polymorphonuclear leukocytes. *Microbiology*, *155*(11), 3500–3508. https://doi.org/10.1099/mic.0.031443-0

Donlan, R. M. (2001). Biofilm Formation: A Clinically Relevant Microbiological Process. *Clinical Infectious Diseases*, *33*(8), 1387–1392. https://doi.org/10.1086/322972

Emma G.S., Lesile R.H., & Tanya L.S. (2017). Pseudomonas aeruginosa LasR·DNA Binding Is Directly Inhibited by Quorum Sensing Antagonists. *ACS Infect. Dis, 3(3), 183–189* https://pubs-acs-org.myaccess.library.utoronto.ca/doi/full/10.1021/acsinfecdis.6b0016

Faure, E., Kwong, K., & Nguyen, D. (2018). Pseudomonas aeruginosa in Chronic Lung Infections: How to Adapt Within the Host? *Frontiers in Immunology*, *9*, 2416. https://doi.org/10.3389/fimmu.2018.02416

Gao, P., Guo, K., Pu, Q., Wang, Z., Lin, P., Qin, S., Khan, N., Hur, J., Liang, H., & Wu, M. (2020). OprC Impairs Host Defense by Increasing the Quorum-Sensing-Mediated Virulence of Pseudomonas aeruginosa. *Frontiers in Immunology*, *11*. https://www.frontiersin.org/articles/10.3389/fimmu.2020.01696

Huffnagle, G. B., Dickson, R. P., & Lukacs, N. W. (2017). The respiratory tract microbiome and lung inflammation: A two-way street. *Mucosal Immunology*, *10*(2), 299–306. https://doi.org/10.1038/mi.2016.108

Joseph P.G, & Helen E.B. (2014). Competition Studies Confirm Two Major Barriers That Can Preclude the Spread of Resistance to Quorum-Sensing Inhibitors in Bacteria. *ACS Chem. Bio.,* 9(10), 2291–2299 https://pubs.acs.org/doi/full/10.1021/cb5004288

Leinweber, A., Weigert, M., & Kümmerli, R. (2018). The bacterium Pseudomonas aeruginosa senses and gradually responds to interspecific competition for iron. *Evolution*, *72*(7), 1515–1528. https://doi.org/10.1111/evo.13491

Lin, C. K., & Kazmierczak, B. I. (2017). Inflammation: A Double-Edged Sword in the Response to Pseudomonas aeruginosa Infection. *Journal of Innate Immunity*, *9*(3), 250–261. https://doi.org/10.1159/000455857

Malhotra, S., Hayes, D., & Wozniak, D. J. (2019). Cystic Fibrosis and Pseudomonas aeruginosa: The Host-Microbe Interface. *Clinical Microbiology Reviews*, *32*(3), e00138-18. https://doi.org/10.1128/CMR.00138-18

Sousa, A. M., & Pereira, M. O. (2014). Pseudomonas aeruginosa Diversification during Infection Development in Cystic Fibrosis Lungs—A Review. *Pathogens*, *3*(3), Article 3. https://doi.org/10.3390/pathogens3030680

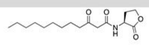
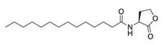
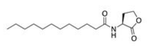
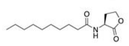
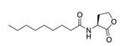
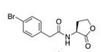
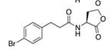
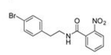
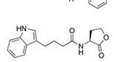
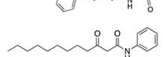
Zhao, X., Yu, Z., & Ding, T. (2020). Quorum-Sensing Regulation of Antimicrobial Resistance in Bacteria. *Microorganisms*, *8*(3), 425. https://doi.org/10.3390/microorganisms8030425

Genetic and solution

QS is related to virulence in pathogenic bacteria, inhibiting QS is a possible antimicrobial strategy.
Studies that supports...

- QS in P. aeruginosa is primarily governed by N-acyl l-homoserine lactone (AHL) autoinducer molecules. The detection of AHLs is mediated by the LuxR-type regulatory proteins LasR and RhlR, which link the binding of specific AHLs to downstream gene expression.
- LasR dimerization and DNA binding are controlled by the binding of its native ligand, N-(3-oxododecanoyl)-l-homoserine lactone (3O-C12-HSL). It has been proposed that LasR complexed with its native ligand and bound to target DNA may additionally interact with RNA polymerase to promote gene expression. (1)
- Investigate the impact of quorum sensing antagonists on LasR·DNA binding by determining whether expression of LasR in the presence of non-native ligands yields soluble protein for further characterization. Full-length LasR was expressed in Escherichia coli grown in media supplemented with each of the ligands listed in the table below. (2)
- Ligand 10 proves that disrupting LasR·DNA binding by QS antagonist strongly inhibit E. coli growth. Ligand 10 is a synthetic derivative of a naturally occurring halogenated furanone and has been proposed to impact LasR-mediated quorum sensing activity in P. aeruginosa

1. https://journals.asm.org/doi/full/10.1128/JB.184.17.4912-4919.2002
2. https://pubs.acs.org/doi/full/10.1021/jacs.5b06728

Ligand		$[M + H]^+$ calculated	$[M + H]^+$ observed
native 3O-C_{12}-HSL		298.19	298.25
1		312.25	312.33
2		284.21	284.33
3		256.18	256.25
4		242.17	242.25
5		298.00	No soluble LasR purified
6		312.02	312.08
7		349.01	349.25
8		287.13	287.17
9		290.20	290.42
10		252.84	Strong negative effect on bacterial growth

- Bacteria secrete chemical molecules to use as sensing signals and the molecules communicate and signal low and high cell densities
- These signals trigger regulatory effects for processes such as virulence, drug resistance, bacterial luminescence, motility, toxin production, and biofilm production

Handout Information, relevant to topic but not critical enough to be on the poster

Why Cystic Fibrosis is of Interest

- One of the leading cause of chronic lung infections
  - Defective CFTR gene leads to mucus buildup and scarred tissue along the respiratory airway
    - Prevent clearance of pathogens trapped in the mucus
  - These conditions allow opportunistic bacteria to grow and establish colonies
- Infections occur more readily stemming from conditions of a genetic disorder

Current CF treatment:

- Antibiotics
- Mucus thinning agents, regular nasal polyp removal to improve ventilation and reduce damage caused by prolonged mucus presence
- Assisted breathing
- Lung replacement surgery

Treatment targeting QS and biofilm formation compared to conventional treatment:

- Targeted to infectious agents (e.g: P.aeruginosa)
- Possible additional treatment avenue for use alongside other conventional methods
- Chronic infections have grown resistant to antibiotics
  - Exchange of resistance genes and protection from external environment facilitated by biofilm formation

4. Biofilm formation and mechanisms script

What is Biofilm

A biofilm is a thin slimy film of bacteria. Through this biofilm bacterias are embedded in this slime, allowing them to stick to each other and to a surface.

The biofilm is a heterogeneous layer of slime, with “channels” that allow transport of nutrients and oxygen to the cells growing within the biofilm.

How is the biofilm created

Microorganisms will first attach to a surface and produce extracellular polysaccharides that result in the formation of a biofilm

Many microorganisms exist by attaching to and growing on living and inanimate surfaces.

In their growth state, the cells develop a biofilm.

Microorganisms will irreversibly attach to and grow on a surface - through cell division - and go on to form microcolonies.

These microorganisms will then produce extracellular polymers that facilitate attachment and matrix formation - aka a biofilm

- These extracellular polymeric substances (EPSs) provide the matrix for the biofilm, and they consist primarily of polysaccharides.

Characteristics of a biofilm

Biofilm colony formations provide a medium for intracellular signals and protection

These organisms that have aggregated together in the biofilm are a colony

- The biofilm allows the unicellular organisms to act as a multicellular organism through the formation of a colony

Biofilms allow for signals to be received quickly

- The signals sent within the biofilm allows for the fortification and protection of the bacteria within the biofilm

The biofilm is a safeguard for the bacterias

- Biofilms are resistant to extreme environment
- Biofilms can protect microorganisms from ultraviolet (UV) radiation, extreme temperature, extreme pH, high pressure, poor nutrients, antibiotics, etc.

* Biofilms are fundamental to colony formation

- Without colony formation, the protection of bacterias and the behaviors of these bacterias can not be established.

Biofilm formation is a leading cause of persistent infections.

Biofilm-associated microorganisms exhibit dramatically decreased susceptibility to antimicrobial agents (when compared to their planktonic counterparts). This is believed to be the cause of infections in patients with indwelling medical devices.

- Lung infections and urinary tract infections are associated with indwelling medical devices and in most cases, are biofilm associated.

Characteristics of the foundation that the microorganisms are building their biofilm on, has shown to have a significant effect on the rate and extent of attachment by microorganisms.

- rougher and more hydrophobic foundations will develop biofilms more rapidly