Haemophilus influenzae metabolic requirements during lung infection

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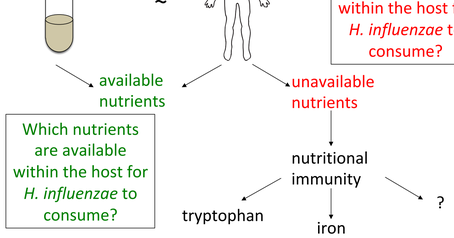
Abstract

Most healthy adults (~75%) carry the bacterium *Haemophilus influenzae* in their nasopharynx without any complications. Nevertheless, *H. influenzae* can spread to other parts of the respiratory tract – the lungs or middle ear – resulting illness and sometimes mortality. *H. influenzae* is an obligate human pathogen; therefore, the human host provides many nutrients *H. influenzae* requires for survival. However, little is known of the metabolism of *H. influenzae* during lung infection. The goal of this study was to answer the following questions: 1) Which nutrients are available for *H. influenzae* to consume in the lung? 2) Which nutrients are unavailable to *H. influenzae* in the lung, potentially because of nutritional immunity (host nutrient sequestration)? To tackle these questions, we used the whole-genome approach, transposon insertion site sequencing (Tn-seq), to determine the fitness of thousands of mutants simultaneously. We subjected the *H. influenzae* Rd transposon mutant library, which contains over 70,000 mutants, to an infection (murine lungs) and a defined metabolic environment (*in vitro* chemically defined medium). Almost half (~48%, 79/166 genes) of the genes *H. influenzae* requires during lung infection are also required during growth in a chemically defined medium. Therefore, *H. influenzae*'s genetic requirements for lung infection are heavily related to metabolism. Based on the nutrients in the chemically defined medium, the genes required for growth in that defined environment, and the genes required in lung infection, we predict that the bacterium's environment in the lung lacks or the host sequesters many nutrients including purines, pyrimidines, tryptophan, valine, alanine, methionine, and serine. Future studies will focus on modifying the chemically defined medium to be more "lung-like" and further characterizing mechanisms of nutritional immunity. Overall, these data provide a more complete view of the nutritional environment of the lung during *H. influenzae* infection, new metabolites involved in nutritional immunity, and potential therapeutic targets.

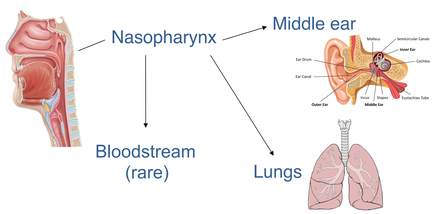
Introduction

The host is a dynamic growth medium



- 1870 – Louis Pasteur – body is culture vessel
- 1960 – E. D. Garber – "The host as a growth medium"
- "The nutritional requirements of the pathogen must be satisfied at the site of inoculation or of localization"
- We must understand bacterial physiology within the host during infection

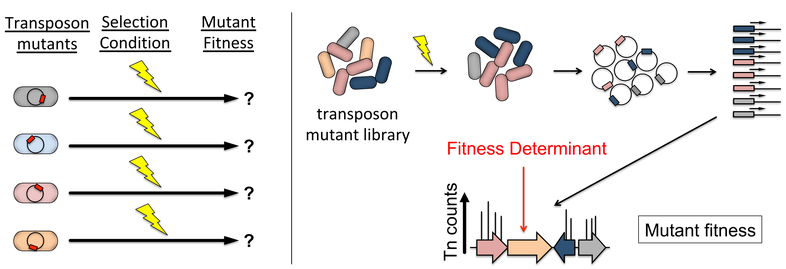
Haemophilus influenzae



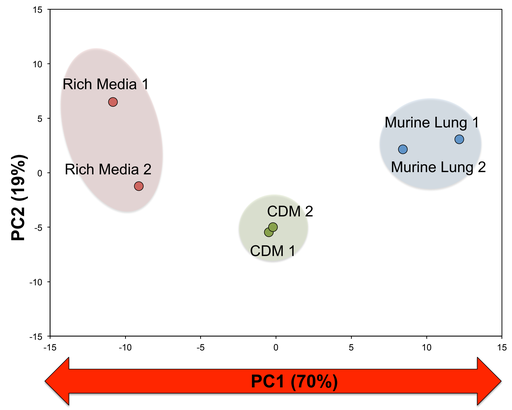
- Obligate human pathogen
- Normally colonizes the human nasopharynx
- Causes infections including otitis media, sinusitis, bronchitis, and pneumonia

Methods

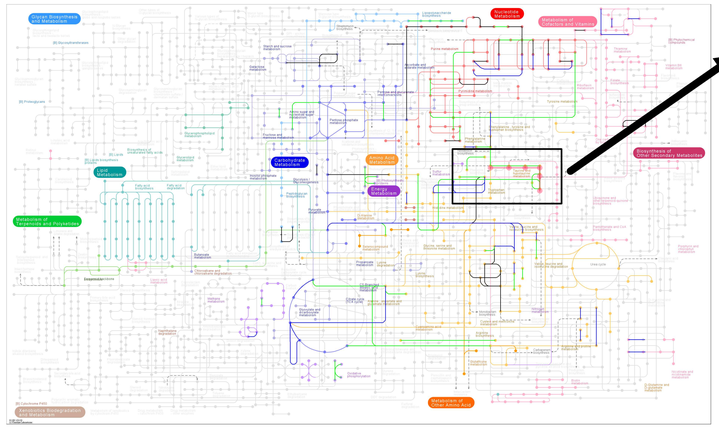
Transposon Insertion Site Sequencing (Tn-seq)



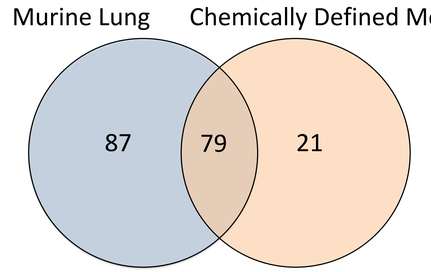
Results



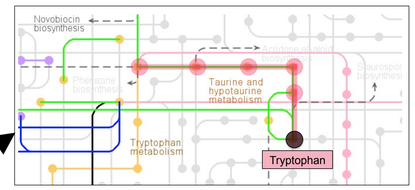
Chemically defined medium is more similar to the lung than rich media



Haemophilus influenzae metabolism



Of the genes *H. influenzae* requires during lung infection, ~48% (79/166) are also required during growth in the chemically defined medium.

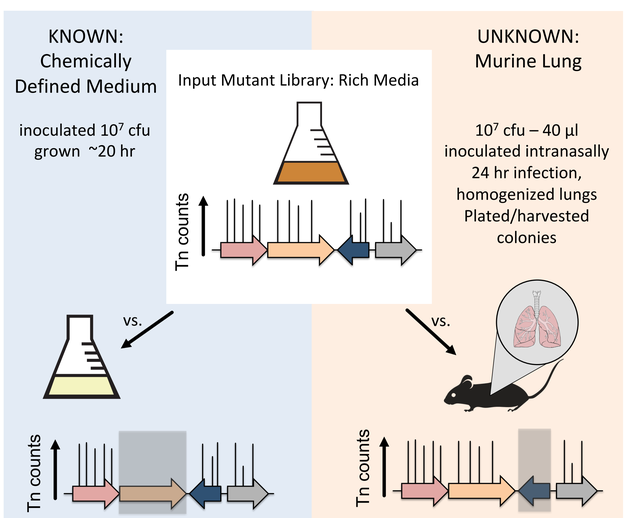


Which metabolites does the host provide for *H. influenzae*?

Metabolites	Available in MM?	Available in lung?
aspartate	yes	yes
glutamate	yes	yes
arginine	yes	yes
lysine	yes	yes
histidine	yes	yes
thiamine	yes	yes
pantothenate	yes	yes
NAD	yes	yes
heme	yes	yes
methionine	yes	no
serine	yes	no
leucine	yes	no
tyrosine	yes	no
cysteine	no	yes
proline	no	yes
pyridoxal	no	yes
purines (A, G)	no	no
pyrimidines (T, C)	no	no
alanine	no	no
asparagine	no	no
tryptophan	no	no
valine	no	no
isoleucine	no	no
phenylalanine	no	no

Methods

CDM Metabolites
aspartate
glutamate
arginine
glycine
lysine
methionine
serine
leucine
tyrosine
histidine
cystine
thiamine
pantothenate
NAD
heme
hypoxanthine
inosine
uracil



Conclusions & Future Directions

Metabolites	How to modify CDM?
aspartate, glutamate, arginine, lysine, histidine, thiamine, pantothenate, NAD, heme	**Keep**
methionine, serine, leucine, tyrosine, cysteine, pyridoxal	**Remove**
proline	**Add**
purines (A, G), pyrimidines (T, C), alanine, asparagine, tryptophan, valine, isoleucine, phenylalanine	**Do not add**

Modify CDM

- CDM is already more similar to the murine lung than rich media
- A few modifications can make CDM more "lung-like."
- These modifications will allow us to study particular pathways of interest, serum susceptibility, and antimicrobial resistance.
- The more modified CDM will provide a more in vivo-like in vitro system, which will allow more control for mechanistic studies.

Study Nutritional Immunity

Candidates for nutritional immunity

methionine, serine, leucine, tyrosine, purines (A, G), pyrimidines (T, C), alanine, asparagine, valine, isoleucine, phenylalanine, tryptophan

Metabolites deemed "unavailable" in the murine lung are candidates for future nutritional immunity studies.

Conclusions

- Our chemically defined medium is more similar to the murine lung than rich media
- Of the genes *H. influenzae* requires during lung infection, ~48% (79/166) are also required during growth in the chemically defined medium
- Fitness data from a chemically defined medium aids in
  - in vivo metabolite prediction
  - improving in vitro systems

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