The Landscape of Adaptive Evolution of a Gut Commensal Bacterium in Aging Mice

Hugo C. Barreto¹, Ana Sousa² and Isabel Gordo¹

¹Instituto Gulbenkian de Ciência, Oeiras, Portugal
²iBiMed, Institute for Biomedicine, Universidade de Aveiro, Aveiro, Portugal
Gut microbiota development during life

*During aging*
- Decline in immune system functioning (*Immunosenescence*)
- Low level of chronic *inflammation*
- *Dysbiosis* of the gut microbiota

Core microbiota

Subdominant taxa

*Bacteroidaceae*

*Lachnospiraceae*

*Ruminococcaceae*

...  

*Odoribacter*

*Oscillospira*

*Enterobacteriaceae*

...  

Biagi 2016, Kundu 2017, Vaiserman 2017, Nagpal 2018
Gut microbiota and aging

- Immunosenesence
- Increased inflammation
- Decline of host functions
- Changing gut ecological environment
- Dysbiosis
- Improper surveillance at the host-microbe interface

Aleman 2019
Gut microbiota and aging

Is the **evolution** of a **bacterial species** different in **old mice**?

Mutagenic / Stressful environment ➔ Increased number of mutations?
How can we study evolution in the gut?

- Streptomycin treatment
- Fecal sample collection
- Gavage with *E. coli*
- 1:1 mixture of YFP / CFP

Emergence of 1 beneficial mutation in YFP background

Emergence of beneficial mutations in both backgrounds: Clonal Interference

Hegreness et al 2006, Barroso-Batista et al 2014

@hugocbarreto
*E. coli* evolution in young animals

**Rapid adaptation** of *E. coli* to the gut of **young animals** and **high level** of **parallelism** (acquisition *gat*-negative phenotype)

Regime of **intense clonal interference**, haplotypes compete for fixation

Barroso-Batista et al 2014

@hugocbarreto
Old mice show higher levels of inflammation

Frailty Index

Frailty index is increased and variable amongst old mice

Young: 2 months, Old: 18 months
Mann-Whitney, ** p < 0.01
Old mice show higher levels of inflammation

**Frailty Index**

- Young: 2 months, Old: 18 months

**Systemic inflammation**

- Mann-Whitney, **p < 0.01**

*Frailty index is increased and variable* amongst old mice
Old mice show higher levels of inflammation

Frailty Index  Systemic inflammation  Gut inflammation

Frailty index is increased and variable amongst old mice

Systemic / Gut Inflammatory environment that can potentially affect *E. coli* evolution

Young: 2 months, Old: 18 months
Mann-Whitney, ** p < 0.01
Old mice have a distinct microbiota composition

**Class level**

![Graph showing microbiota composition before and after treatment](image)

Differentely represented taxa between young and old mice

Before treatment: 17 taxa

After treatment: 3 taxa

**After antibiotic treatment and colonization with E. coli,** the **differences** in microbiota composition **became less pronounced** but **still persisted.**

Young: 2 months, Old: 18 months

@hugocbarreto
*E. coli* evolves rapidly in the gut of old mice

Markers diverge around the same time as in young animals

*E. coli* evolves as rapidly as in young animals

Young: 2 months, Old: 18 months
The spread of loss of ability to consume galactitol is delayed in old mice

Other beneficial mutations may be occurring earlier

Young: 2 months, Old: 18 months
Mann-Whitney, * p < 0.05, ** p < 0.01, *** p < 0.001
The mutational landscape is different between young and old mice

A small percentage of *E. coli* adaptive targets are common between young and old mice.

Mice cluster by age (*p* = 0.002), supporting that the mutational landscape is different.
Mutations observed in *E. coli* are due to selection.

**Number of mutations**

- Number of segregating mutations per animal is **higher** in old mice.

Young: 2 months, Old: 18 months
Mutations observed in *E. coli* are due to selection

Number of segregating mutations per animal is higher in old mice.

No differences in the mean mutation rate
Mutations observed in *E. coli* are due to selection.

**Number of mutations**

Number of segregating mutations per animal is **higher** in **old** mice.

**Mutation rate in vivo**

No differences in the mean mutation rate

**Negative correlation** between mutation rate and number of mutations

Young: 2 months, Old: 18 months
Signature of *E. coli* evolution in old mice: Stress response
Signature of *E. coli* evolution in old mice: Stress response

Involved in metabolism

Young: 2 months, Old: 18 months

@hugocbarreto
Signature of *E. coli* evolution in old mice: Stress response

Young: 2 months, Old: 18 months
Signature of *E. coli* evolution in old mice: Stress response

- Young: 2 months, Old: 18 months

**Involvement in metabolism**
- *focA/ycaO*
- *kdgR*
- *dcuB/dcuR*
- *srlR*
- *tdcA/tdcR*
- *gat-operon*

**Phosphate Starvation**
- *ddlA/iraP*
- *mngB/cydA*
- *puuE*
- *lrhA*
- *iscR*
- *selA*
- *spoT*

**Nitric oxide stress**
- *Osmotic and heat stress*
**High levels of Zinc(II)**
- *Oxidative stress*
- *Stringent response*
- *Iron homeostasis*

@hugocbarreto
Strong selection dominates the evolutionary process of *E. coli* in the aging gut

*iscR* dynamics

*iscR* emerges at the same time as the *gat*-negative phenotype (Day 2)

*iscR* has an estimated selective effect of 17% per generation, measured from 2-4 days

Young: 2 months, Old: 18 months
Strong selection dominates the evolutionary process of *E. coli* in the aging gut

*IrhA* dynamics

*IrhA* selective effect at least as high as that of the *gat*-negative phenotype (~7% per generation)
Selection for motility during the evolutionary process of *E. coli* in the aging gut

Unpaired t-test, *p < 0.05, **p < 0.01, ***p < 0.001.
**Summary**

**Rapid evolution of E. coli**

**Young mice**
- **Rapid emergence and Strong selection for gat-negative**
- Targets metabolism genes

**Old mice**
- **Delayed emergence of gat-negative**
- Targets mostly stress response genes
- **Strong selection for iscR and lrhA**
- **Selection for motility**
Acknowledgments

Supervisor
– Isabel Gordo, IGC

Co-Supervisor
– Ana Margarida Sousa, iBiMED Uni Aveiro

IGC Rodent Facility
IGC Genomics Unit

Evolutionary Biology Group

INSTITUTO GULBENKIAN DE CIÊNCIA

FCT Fundação para a Ciência e a Tecnologia
ONEIDA AN OMICS NETWORK

Lisboa2020 Portugal 2020 EUA