Fecal virome in decompensated liver cirrhosis patients

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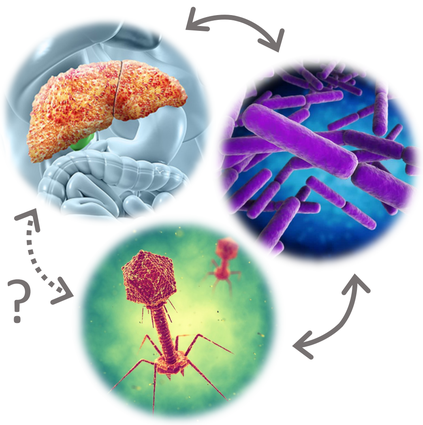
INTRODUCTION

Phages play a crucial role in the complex gut microbial ecosystem through their interactions with gut bacteria. The gut microbiota is implicated in the development of **decompensated cirrhosis (DC)**, a disease causing 1.2 million deaths per year. However, the mechanisms behind the progression towards **acute-on-chronic liver failure (ACLF)**, a severe complication of DC, are not yet understood. While **bacterial translocation and bacterial infections** have been reported as precipitating factors, large interindividual differences remain. It is hypothesized that the viral fraction of the human gut microbiome (**virome**) could be involved here.



METHODS

MUCOSA-PREDICT cohort
- 93 decompensated liver cirrhosis patients
- 309 fecal samples*

* 292 samples remained after normalisation

NetoVIR protocol
- viral enrichment
  - centrifugation
  - filtration
  - nuclease treatment
- DNA & RNA extraction
- random amplification
- library preparation
- Illumina sequencing

Bioinformatic processing
- read trimming
- per-sample *de novo* assembly (> 1 kb)
- cross-sample clustering (95% ID – 85% cov)
- viral identification**
- abundance & normalisation (70% coverage)
- bacterial host & lifecyle prediction***

** **eukaryotic viruses**: protein homology (DIAMOND) **or** nucleotide homology with (BLASTn)
phages: Virsorter2 signal with CheckV completeness estimate **or** *Inoviridae* protein homology (DIAMOND)
*** **phages > 50% complete**: prophage, CRISPR, tRNAs, PHIST, RaFAH & BACPHLIP (DBs: bacterial MAGs + UHGG db)

RESULTS & DISCUSSION

Higher phage diversity and attenuation of virulent phage dominance in ACLF

Phage diversity is increased in patients with ACLF compared to patients without ACLF (**Fig. 1A**) as well compared to their own pre-ACLF sample (if available) **(Fig. 1B)**. **Phage** diversity has already been associated with severity of liver cirrhosis [Bajaj *et al.* 2020]. Virulent phages dominate phageomes of individuals without ACLF, while this dominance is attenuated in individuals with ACLF **(Fig. 1C)**. This shift from a virulent-dominated towards a balanced virulent-temperate phage community is also observed intra-individually when comparing the pre-ACLF sample of a patient to its own ACLF sample (**Fig. 1D**). The more balanced virulent-temperate phage community in ACLF samples is accompanied by both an increased **temperate phage** diversity as well as an increased virulent phage diversity (**Fig. 1E**), both contributing to the increased global **phage** diversity. The increased diversity of **temperate phages** in ACLF might potentially be caused by the inflammation characteristic for ACLF, which could trigger the induction of prophages.

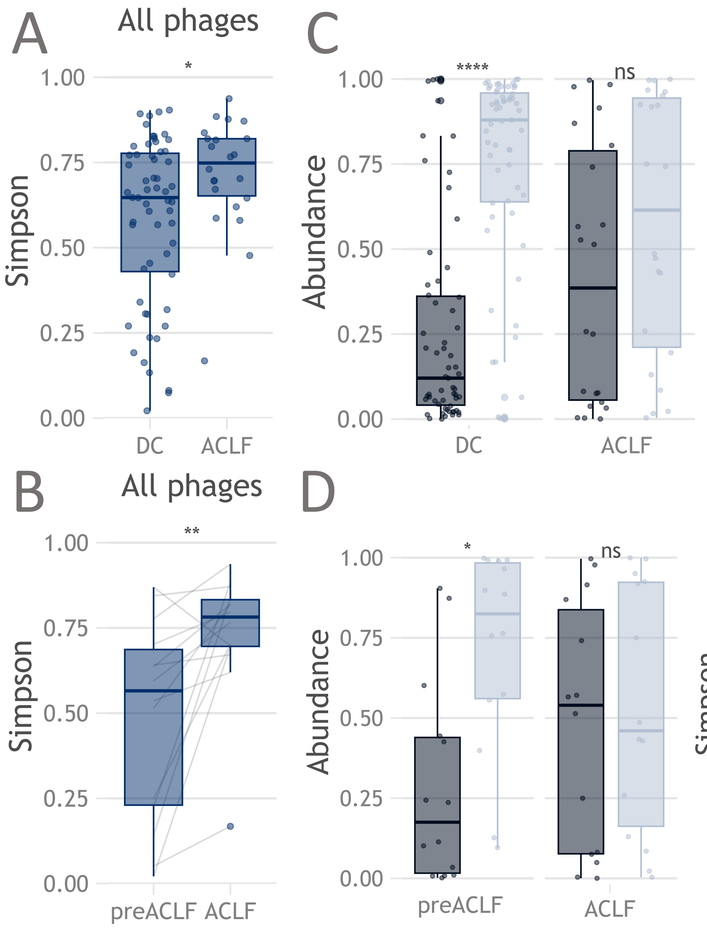


Figure 1: Diversity & abundance of phages in ACLF.
Simpson alpha-diversity (A, B, E) and relative abundance (C & D) of all phages (A & B) and temperate phages vs. virulent phages (C, D, E).
Inter-individual comparisons between patients without and with ACLF (n = 59 vs. n = 22) (A & C) or intra-individual comparisons of ACLF patients to their own pre-ACLF sample (n = 14) (C, D, E).

Enterococcus B-infecting phages associated with bacterial infection

As bacterial infection is one of the major precipitating events for the development of ACLF, we tried to identify groups of phages infecting a specific bacterial host that are associated with bacterial infection in this cohort. Phages infecting one bacterial genus, ***Enterococcus B***, were found to be more abundant and more prevalent in samples with bacterial infection (Wilcox. adj. p = 0.007 – Log2FC = 3.5 and Fisher adj. p = 0.01 – OR = 3.5; **Fig. 2A**). Also the following KEGG pathways were found more often in samples with bacterial infection: biotin metabolism, fatty acid biosynthesis and aminobenzoate degradation (Fisher adj. p < 0.05; **Fig. 2B**). The ***Enterococcus*** genus has previously been associated with MELD score (measure of severity of chronic liver disease) [Bajaj *et al.* 2020]. Moreover, an increased abundance of phages infecting this genus have been observed in patients with alcoholic hepatitis [Jiang *et al.* 2020]. Of note, a specific phage targeting cytolysin-positive ***E. faecalis*** has been used to attenuate alcoholic liver disease in mice [Duan *et al.* 2019]. These results indicate ***Enterococcus*** bacteria and their phages might play crucial roles in the progression of liver disease.

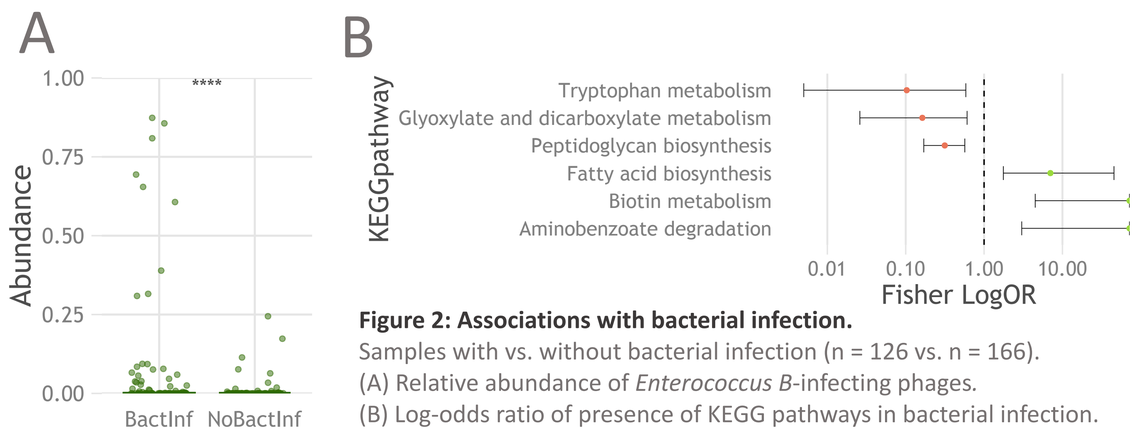


Figure 2: Associations with bacterial infection.
Samples with vs. without bacterial infection (n = 126 vs. n = 166).
(A) Relative abundance of *Enterococcus B*-infecting phages.
(B) Log-odds ratio of presence of KEGG pathways in bacterial infection.

Lactococcus A-infecting phages associated with preACLF and mortality

Lactococcus A-infecting phages are more abundant patients with preACLF compared to patients with decompensated cirrhosis (Wilcox. adj. p = 0.003 - Log2FC = 7; **Fig. 3A**). **Lactococcus**-infecting phages were previously shown to be enriched in alcohol use disorder [Hsu *et al.* 2022] and depleted in alcoholic hepatitis [Jiang *et al.* 2020] and NAFLD [Lang *et al.* 2020]. In this study, presence of ***Lactococcus A***-infecting phages were also linked to higher mortality (log-rank test adj. p = 0.014; **Fig. 3B**). Of note, this association is likely confounded by the higher abundance of ***Lactococcus A***-infecting phages in preACLF patients, a more severe clinical course of acute decompensation. Apart from ***Lactococcus A***-infecting phages, also ***Flavonifractor***-infecting phages were associated with higher mortality (log-rank test adj. p = 0.086; **Fig. 3C**). More patients harboured ***Flavonifractor***-infecting phages in their gut virome compared to ***Lactococcus A***-infecting phages.

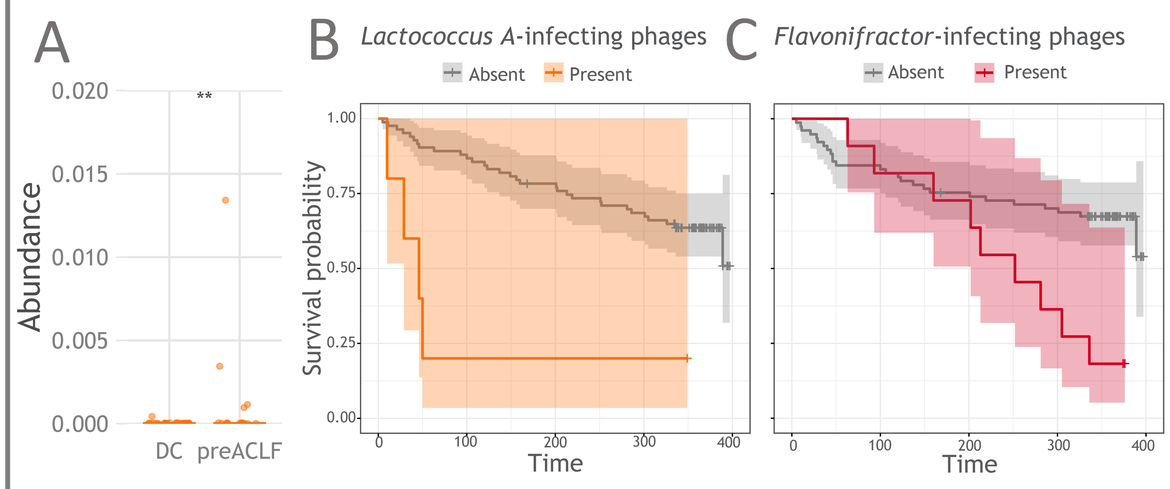


Figure 3: Associations with preACLF and mortality.
(A) Relative abundance of *Lactococcus A*-infecting phages in patients with stable and unstable decompensated cirrhosis (DC; n = 62) vas. preACLF patients (n = 21).
(B & C) Survival curves of patients with and without *Lactococcus A* and *Flavonifractor*-infecting phages.

CONCLUSION

A higher phage alpha-diversity is associated with a more severe clinical course: progression towards ACLF. Progression towards ACLF also causes the phageome to be less dominated by virulent phages, potentially through the induction of prophages. Although individual phages generally have a low prevalence in the human population, grouping them by their host proves to be valuable to identify specific groups of patients such as those with bacterial infection, preACLF or with higher mortality. This can potentially be relevant in light of personalized treatment strategies. This first exploration of the virome in DC, in combination with other microbiome and host-related multi-omics research performed as part of the MICROBPREDICT project, will provide important foundations for a better understanding of the mechanisms underlying this disease.

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