Synthesizing the human gut microbiome

Joint Graduate Seminar

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Outline

01 What is microbiome synthesis

02 Why to synthesize microbiome

03 How to synthesize gut microbiome from scratch

04 *In vitro* systems

05 The promising applications when combining the two techniques

06 Take home messages

What is microbiome synthesis

- Definition:

  The approach of mixing or co-culturing multiple taxa under well-defined conditions to mimic the structure and function of a microbiome [1]

- First attempt to create a synthetic community in 1965:

  A defined mixture of six strains isolated from normal mice to study the association of the organisms and germ-free mice [2]

ASSOCIATION OF GERMFREE MICE WITH BACTERIA ISOLATED FROM NORMAL MICE*

By RUSSELL W. SCHAEDLER, M.D., RENÉ DUBOS, PH.D., AND RICHARD COSTELLO, PH.D.

(*From The Rockefeller Institute*)

PLATE 11

(Received for publication, March 9, 1965)

Several species of animals have been raised and made to reproduce under germfree conditions. While the animals so produced seem to have a normal life span, they exhibit histological, anatomical, and physiological characteristics which differentiate them from animals raised under conventional conditions. The very abnormalities of germfree life have thus yielded useful knowledge concerning the role played by microbial activities in the development and physiological performance of higher animals.

The availability of bacterial cultures isolated from the gastrointestinal tract of normal mice has given us the opportunity to study the consequences of associating these organisms with germfree mice. The present paper describes the results of the first long range experiments in this series. Our observations were focussed on (*a*) the fate of the bacteria in the various organs of the gastrointestinal tract following administration by feeding, and (*b*) the effect of bacteria so administered on the size of the cecum.

[1] Shayathan *et al*. 2022. Front Agron
[2] Shaedler *et al*. 1965. J Exp Med

Why do we want to synthesize microbiome

- There are substantial linkages between alterations of the gut microbiome (dysbiosis) and many diseases and conditions, such as obesity, diabetes mellitus, IBD, and colorectal cancer.

- To study the mechanism and causality in the microbiome, inoculate the gnobiotic mice with:

(1) Complete, undefined communities (i.e. fecal microbiota transplantation, FMT)

(2) Incomplete but defined communities (i.e. synthetic communities)

Cheng *et al*. 2022. Cell

Complete, undefined communities – FMT

- Classic studies to show causation: research on obesity in germ-free mice

Turnbaugh *et al*.– cecum-derived microbiota from obese mice

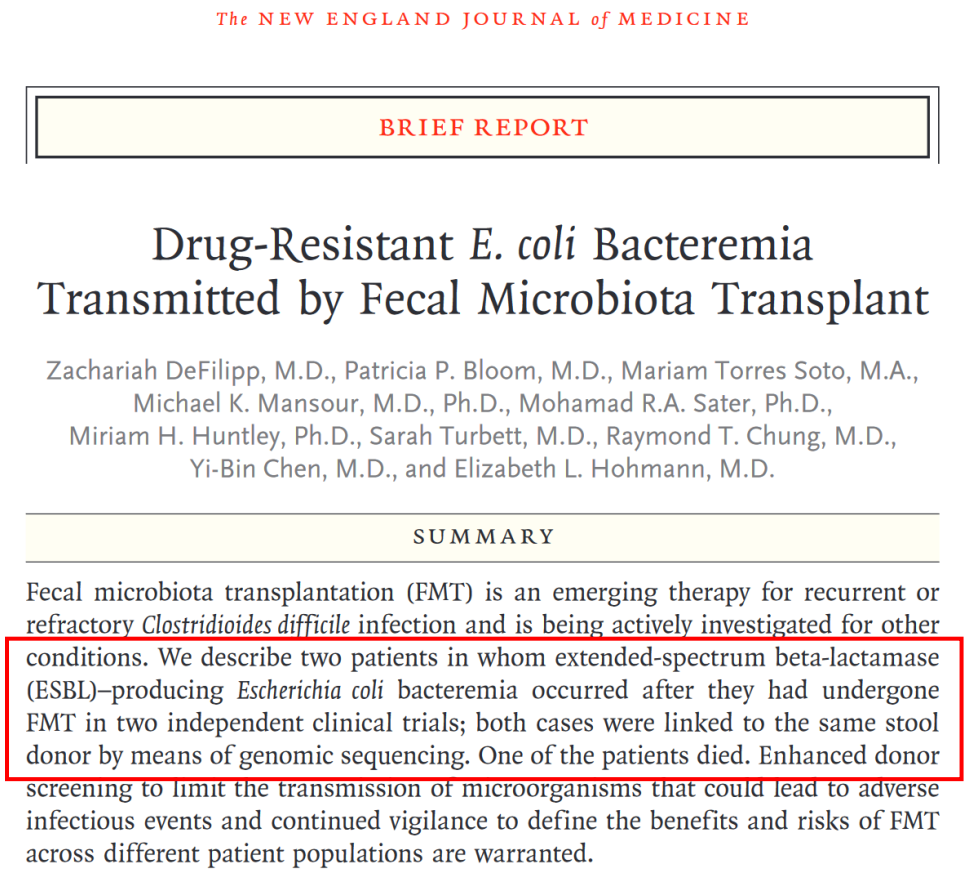
Ridaura *et al*.– FMT from obese humans

- Clinical treatment: FMT for the prevention of recurrence of *Clostridioides difficile* infection (CDI)
- Limitations of FMT:

The screening of donors

Undesired outcomes

Difficult to "fractionate" an undefined community, making it challenging to discover which species are involved in a phenotype of interest



The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Drug-Resistant *E. coli* Bacteremia Transmitted by Fecal Microbiota Transplant

Zachariah DeFilipp, M.D., Patricia P. Bloom, M.D., Mariam Torres Soto, M.A., Michael K. Mansour, M.D., Ph.D., Mohamad R.A. Sater, Ph.D., Miriam H. Huntley, Ph.D., Sarah Turbett, M.D., Raymond T. Chung, M.D., Yi-Bin Chen, M.D., and Elizabeth L. Hohmann, M.D.

SUMMARY

Fecal microbiota transplantation (FMT) is an emerging therapy for recurrent or refractory *Clostridioides difficile* infection and is being actively investigated for other conditions. We describe two patients in whom extended-spectrum beta-lactamase (ESBL)–producing *Escherichia coli* bacteremia occurred after they had undergone FMT in two independent clinical trials; both cases were linked to the same stool donor by means of genomic sequencing. One of the patients died. Enhanced donor screening to limit the transmission of microorganisms that could lead to adverse infectious events and continued vigilance to define the benefits and risks of FMT across different patient populations are warranted.

Turnbaugh *et al*. 2006. Nature; Ridaura *et al*. 2013. Science
DeFilipp *et al*. 2019. N Engl J Med
Wang *et al*. 2019. J Formos Med Assoc

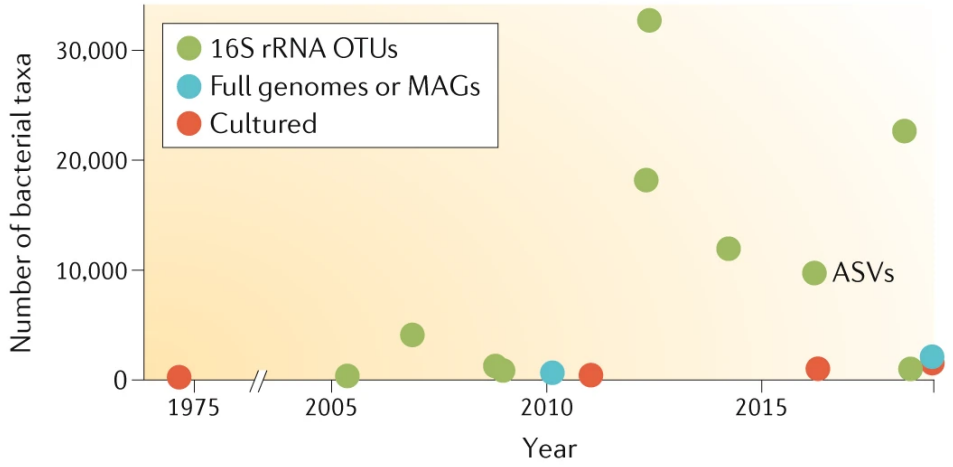
Incomplete but defined communities – synthesized microbiome

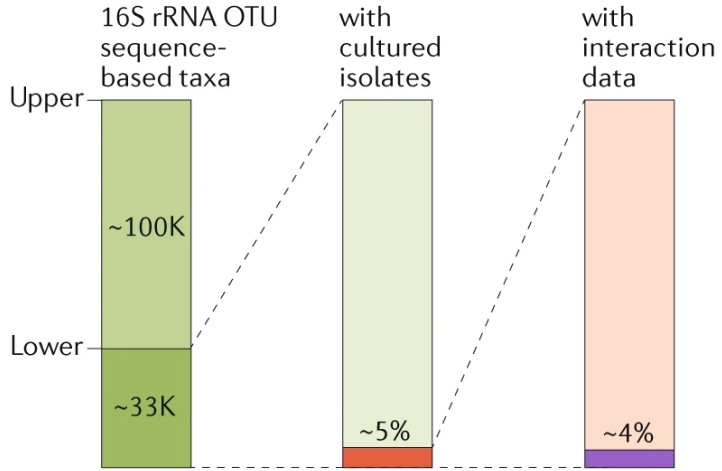
- Prerequisites for the approach:

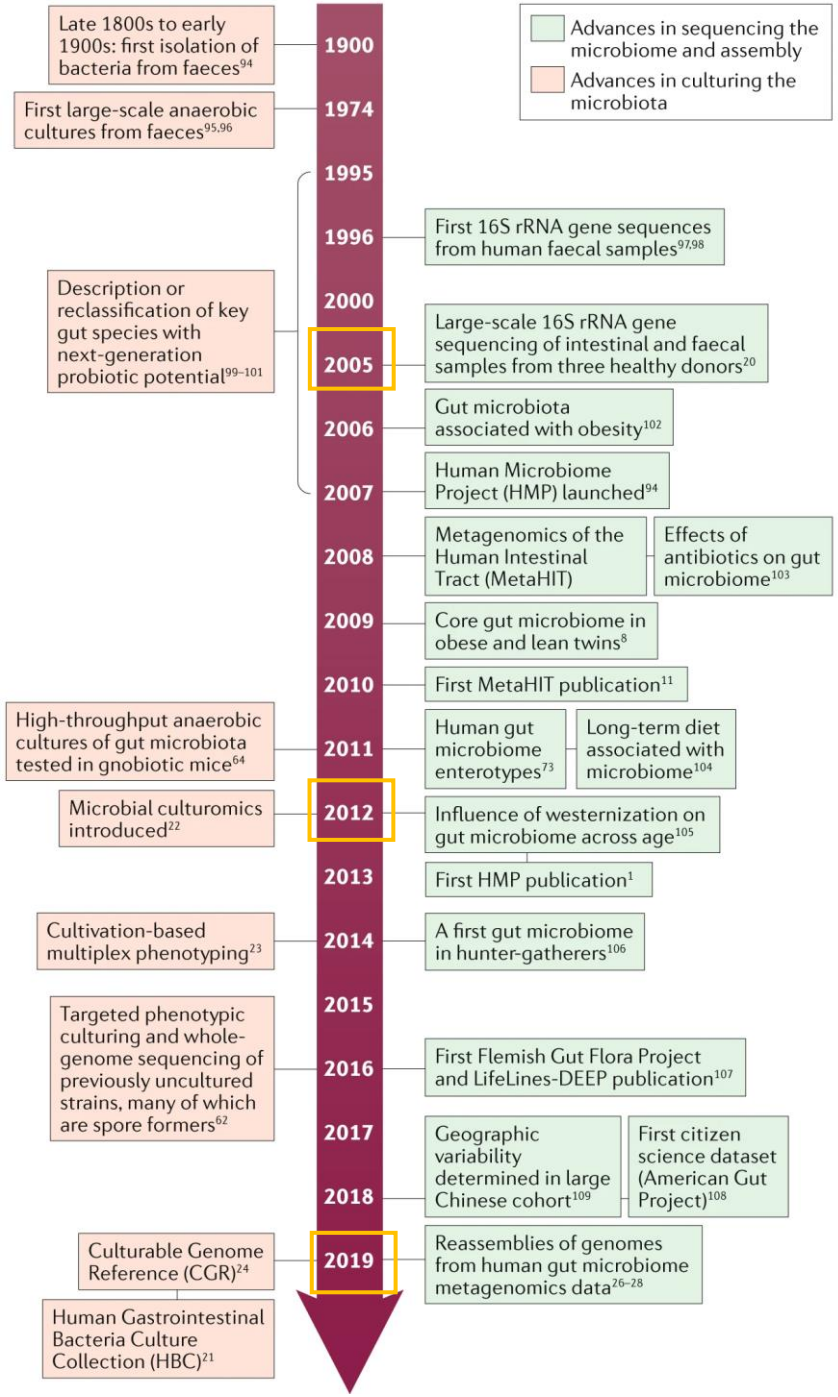
1. Knowledge of the diversity and composition of the targeted microbial community
2. Public databases of reference strains
3. Advanced techniques in cultivation
4. Rapid identification of taxonomy: 16S rRNA gene sequencing or metagenomics
5. Accurate assessment of abundance of the strains: metagenomics or qPCR

Vrancken *et al*. 2019. Nat Rev Microbiol

Timeline of select highlights in the sequencing and culturing of the human gut microbiota

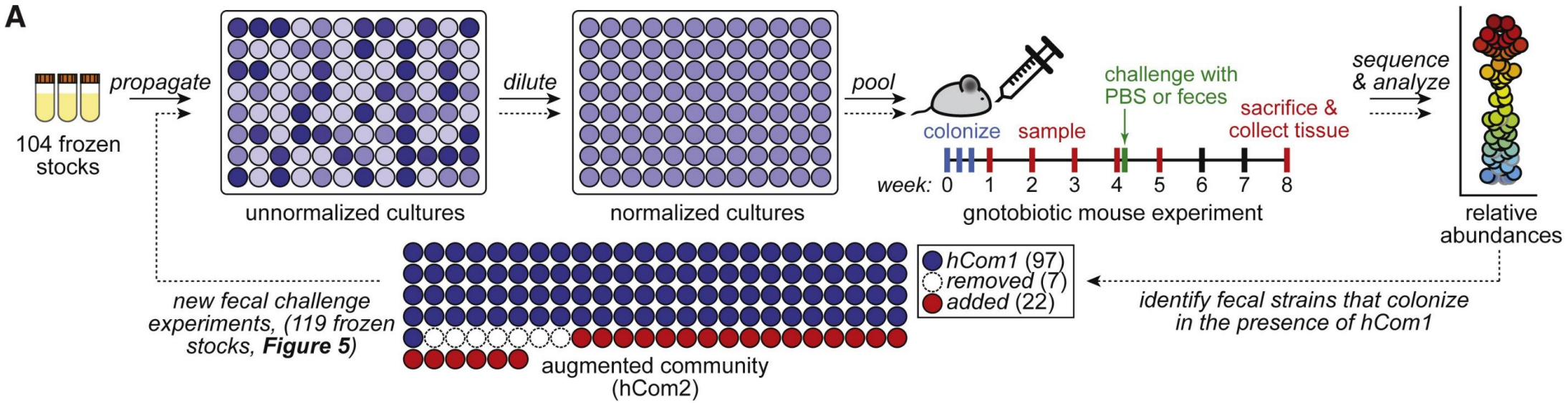






Vrancken *et al*. 2019. Nat Rev Microbiol

The first synthetic human microbiome entirely from scratch



Two communities were synthesized:

hCom1: a shortlist (104 strains) of the most prevalent bacteria found in most people based on the prior human microbiome research (the HMP project)

hCom2: a more complete one (119 strains) and derived from hCom1.

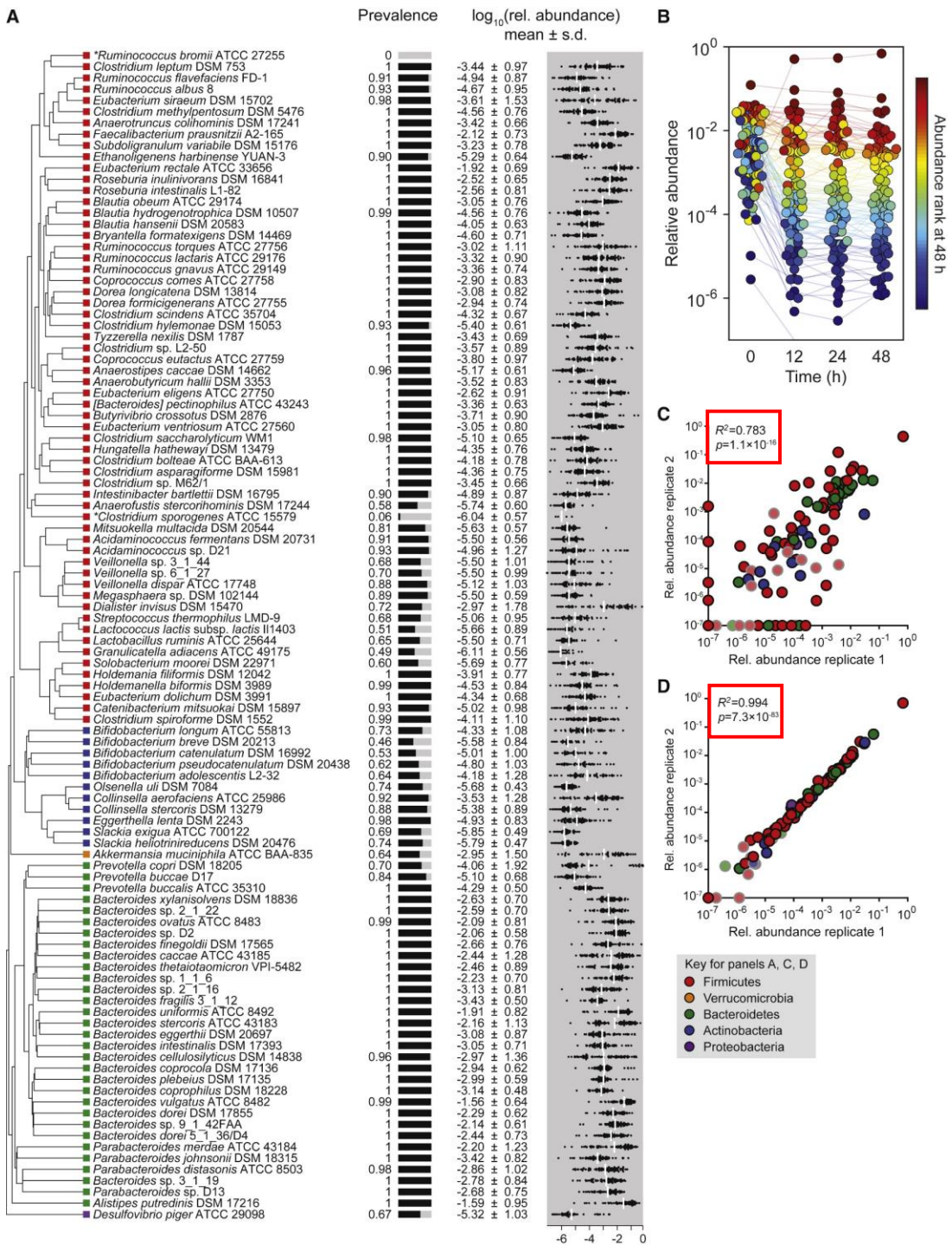
The plan: 1. colonize germ-free mice with hCom1; 2. wait 4 weeks; 3. challenge it with human fecal samples

Expected outcome: certain strains from fecal samples would fill in the niches which were left open in hCom1. Then the invaders can be fished out.

Cheng *et al*. 2022. Cell

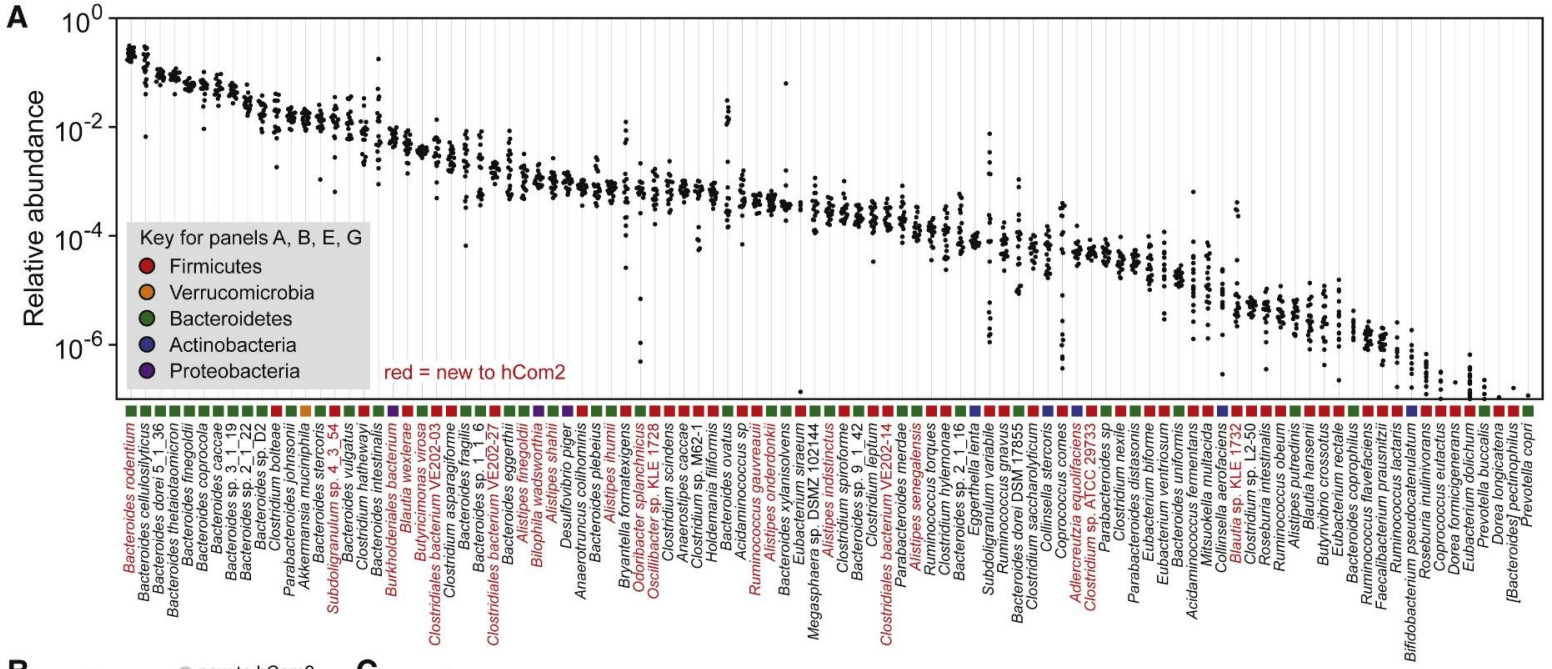
A complex gut microbial community – hCom1

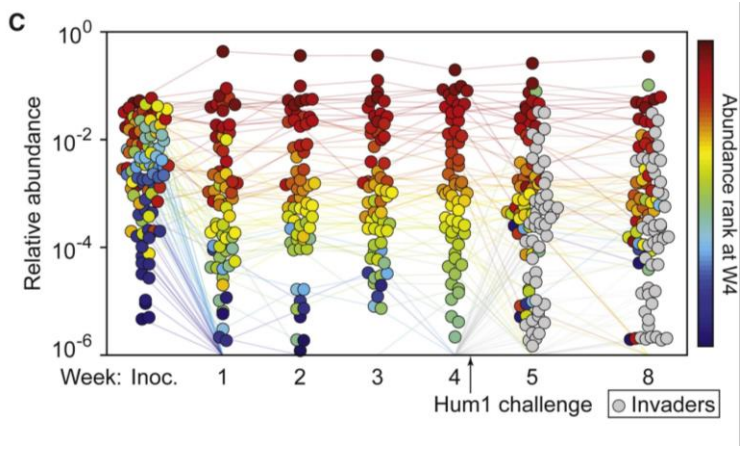
A. The phylogenetic tree of hCom1 based on the multiple alignment of 40 conserved ssingle-copy marker genes. Prevalence: the fraction of subjects in which the strain is detected.
B. Stability test. The relative abundances of strains in the community remained largely stable through 48 h.
C. Biological replicates at 48h. Communities generated from two inocula on different days.
D. Technical replicates at 48h. Communities generated from the same inoculum.

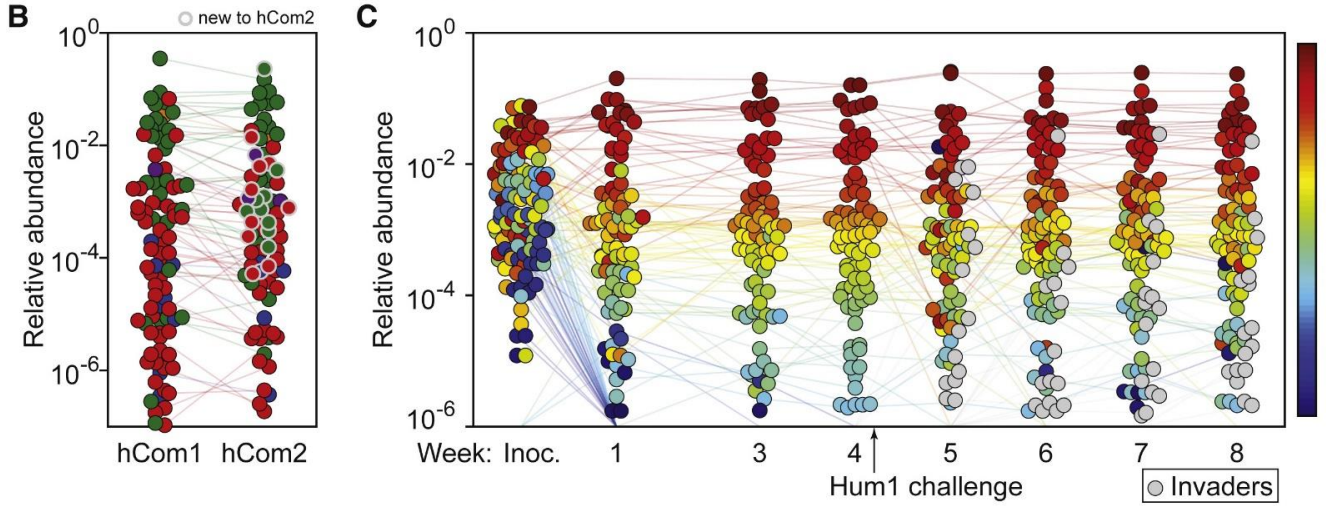


An augmented community – hCom2

A. The relative abundances of each strain in hCom2, with the newly added taxa labelled in red
B. The community structure of hCom2 is similar to hCom1
C. When compared with the post-challenge community of hCom1 (the left), hCom2 is more stable, more resilient and less affected by the invaders

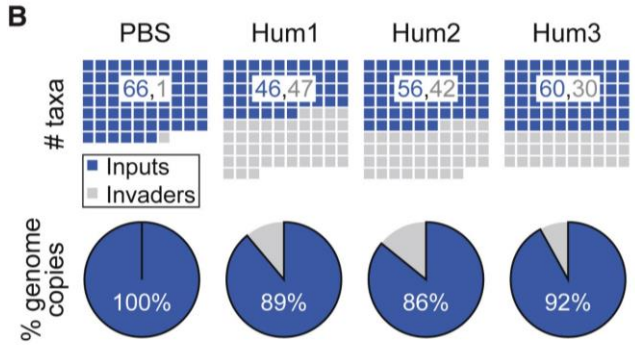






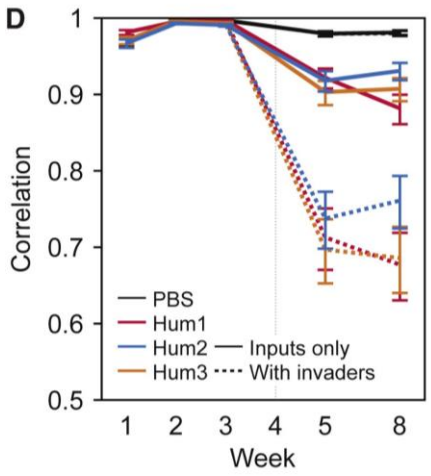
Cheng *et al*. 2022. Cell

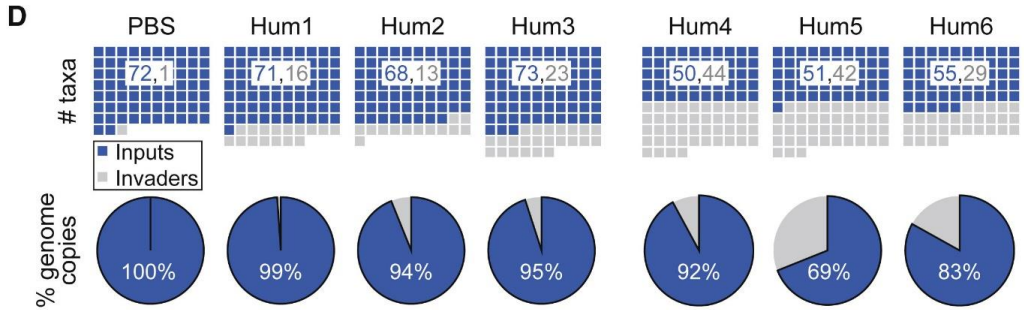
Comparison between hCom1 and hCom2

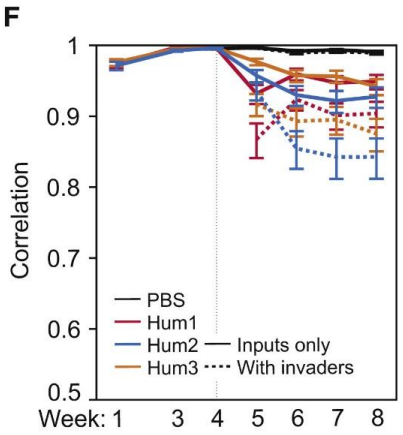


Results of hCom1 when challenged with human fecal samples

1. Lower similarity to fecal samples compared to PBS-control
2. The relative abundances of hCom1 species present post-challenge are highly correlated with pre-challenge levels





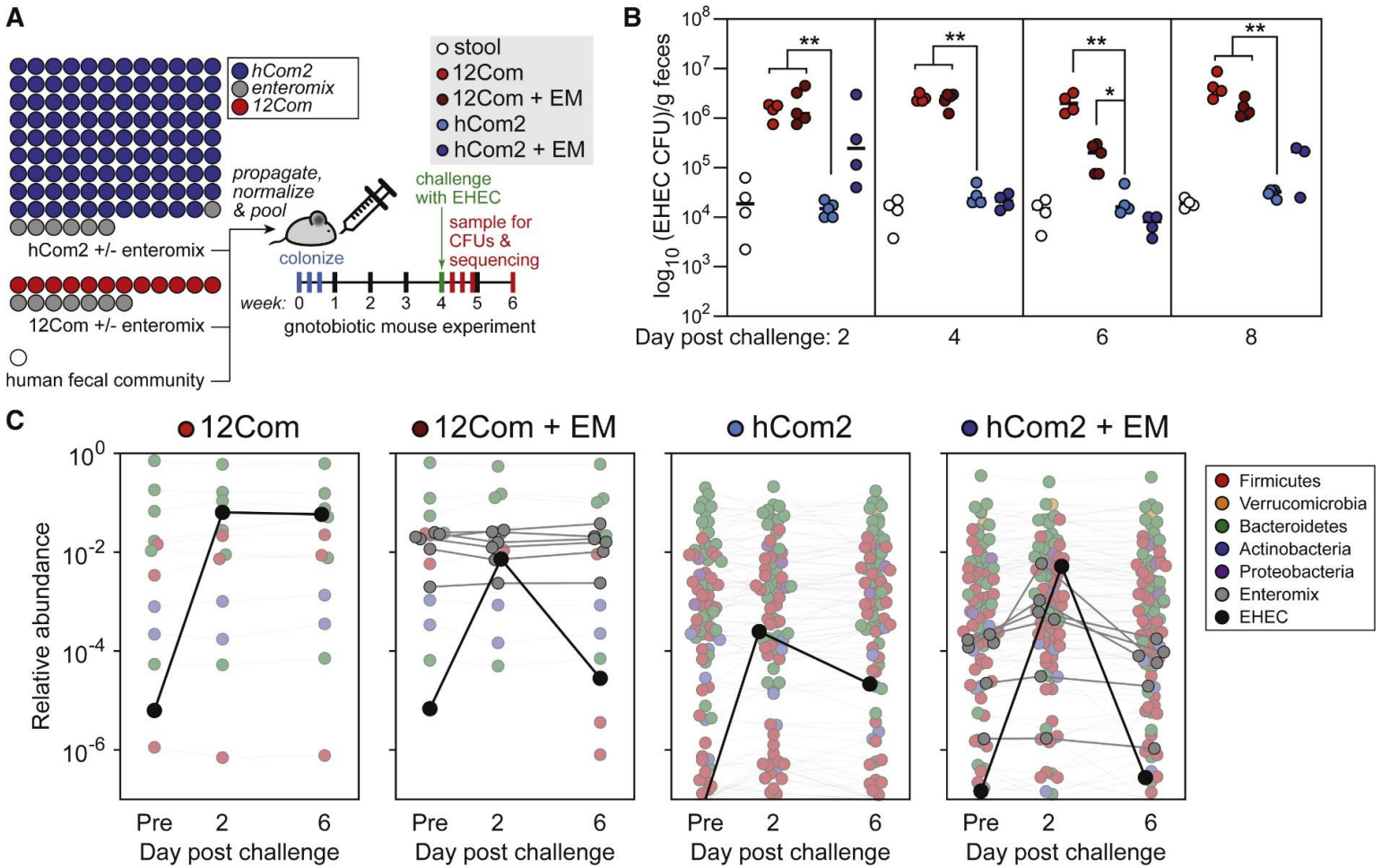


Results of hCom2 when challenged with human fecal samples

1. Similarity to Hum 1-3 increased
2. Different to unrelated fecal samples Hum 4-6
3. Correlation is less effected by invaders

Cheng *et al*. 2022. Cell

hCom2 exhibits colonization resistance against enterohemorrhagic *E. coli* (EHEC)



Colonization resistance: a phenomenon that resident species exclude newcomers

Comparing the ability of colonization resistance with:

1. Stool
2. A 12-member synthetic community – 12Com
3. hCom2
4. Adding enteromix to 12Com and hCom2 – 12Com + EM and hCom2 + EM

EM: Enteromix, a mixture of seven commensal Enterobacteriaceae strains

Limitation of the methodology

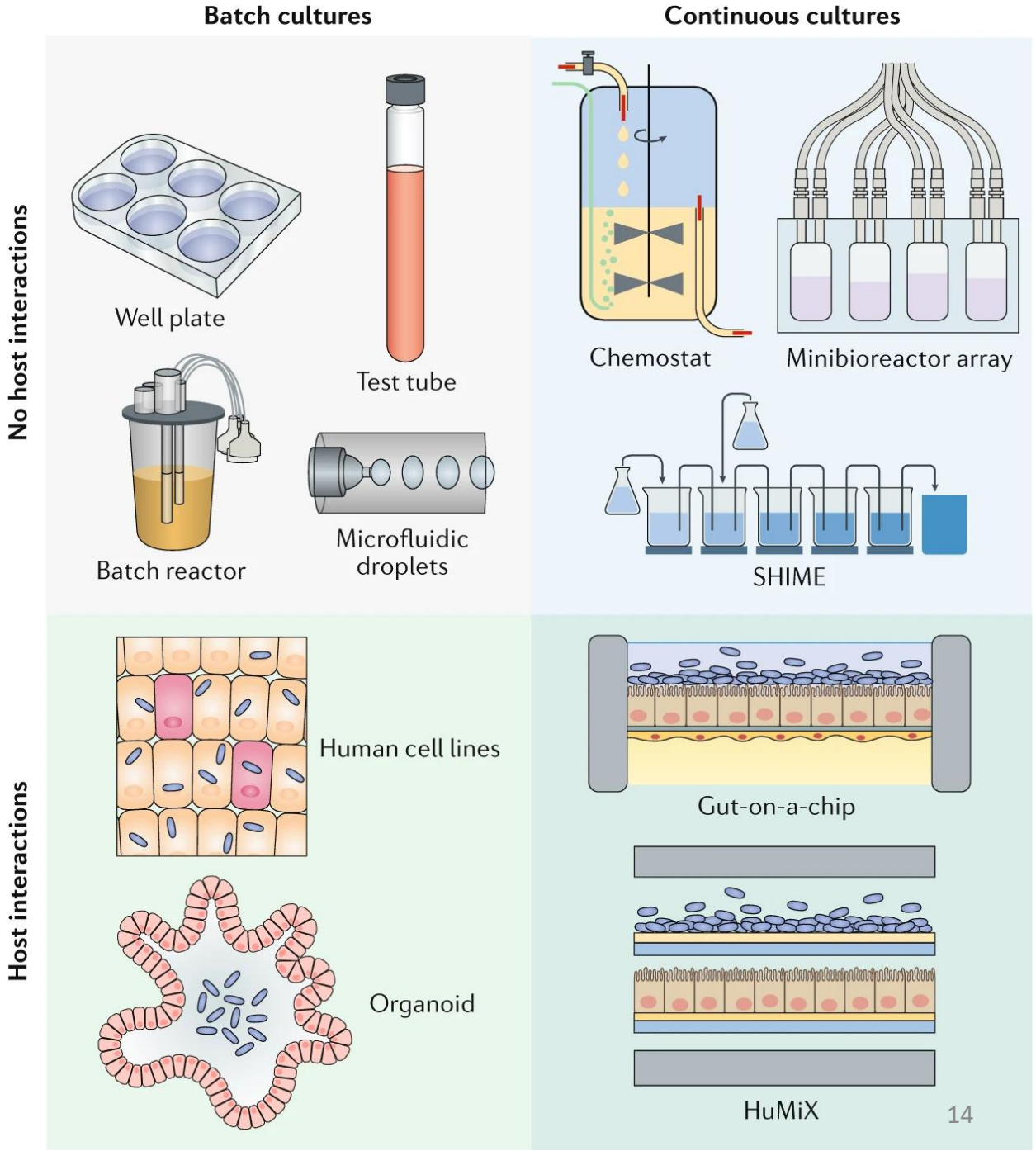
- Less stable to challenge with unrelated fecal communities.
- It is unclear how many more bacterial strains (or other components) may be necessary to model the full functional capacity of a native human microbiome. Prior estimates of the number of species in a typical human microbiome range from ~150–300.
- Strain-level variation among communities underlies some of the phenotypic differences in the host caused by the microbiome. hCom2 represents just one consortium of strains; therefore, neither hCom2 nor any other single community can model the impact of strain-level variation on host phenotype.

Combine *with In vitro* systems

Microfluidic platform: It allows to perform a set of fluidic unit operations that are enabled by a set of fluidic elements

Gut-on-a-chip: Chips lined with living human gut cells and their tiny fluidic channels reproduce blood and/or air; only allow microbes to grow under aerobic conditions

HuMiX: Human–microbial crosstalk, a modular microfluidics-based human–microbial co-culture platform; can create anaerobic conditions



Vrancken *et al*. 2019. Nat Rev Microbiol

The promising applications

- The diet-microbiome interactions: using patient-derived microbiome and host cells
- The drug-microbiome interactions: interconnection of gut-on-a-chip and liver-on-a-chip for predictive chemoinformatic analysis and pharmacokinetic modelling of the microbiome-dependent serum levels
- The microbiome-based precision and personalized medicine
- The mechanistic studies to validate theoretical hypothesis from ‘omics’ observations and microbiome-target intervention in diseases

Moossavi *et al*. 2022. Trends Microbiol

Take home message

- It’s essential to synthesize microbiome for both scientific research and clinical practice

- To synthesize microbiome, the prerequisites include:
  knowing the composition of the target
  databases of reference strains
  cultivation techniques
  identification techniques of taxonomy and abundances

- A methodology to synthesize the human gut microbiome from scratch

- More applications when combining synthesized microbiome with *in vitro* systems