microbial biotechnology

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Crystal Ball

Sustainable Microbiome: a symphony orchestrated by synthetic phages

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Summary

We are surrounded by microbes, mostly bacteria and their viruses or phages, on the inside and outside of our bodies. These bacteria in constant interactions with phages are regulating multiple functions critical to our health. Luckily, they are amenable, but we need precise tools for their safe manipulation and improving human health. Here, we argue that recent advances in single-cell technologies, culturomics and synthetic biology offer exciting opportunities to create these tools as well as revealing specific phages–bacteria interactions in the body.

Introduction

The human body harbours trillions of microbes that are collectively known as microbiota or the microbiome, a term which can also refer to their genetic pool (Khan Mirzaei, khan et al., 2020; Khan Mirzaei, xue *et al*., 2020). The human microbiome consists of bacteria, viruses, archaea, fungi and parasites (Huttenhower *et al*., 2012; Khan Mirzaei, khan *et al*., 2020). The large-scale analysis of these complex communities over the past two decades has revealed their structure, function and diversity as well as their active role in regulating multiple host functions, including circadian rhythms (Kuang *et al*., 2019), metabolism (Pedersen *et al*., 2016) and immunity (Zheng *et al*., 2020). They can also promote our health by producing essential metabolites, such as short-chain fatty acids and vitamins, like cobalamin. Yet, some gut bacteria can be harmful for example *Enterococcus faecalis* which produces cytolysin, a toxin that causes liver injury, while (Duan *et al*., 2019) Trimethylamine-*N*-oxide, a by-product of gut bacteria metabolism, is associated with cardiovascular disease (Tang *et al*., 2013). Imbalance in the bacterial community with the loss of overall diversity, referred to as dysbiosis, is associated with several human diseases or conditions, including inflammatory bowel diseases (IBD) (Franzosa *et al*., 2019), colorectal cancer (CRC) (Wong *et al*., 2017), obesity (Turnbaugh *et al*., 2006), type 2 diabetes (Wu *et al*., 2017), autism (Sharon *et al*., 2019), asthma and allergy (Stokholm *et al*., 2018). Dysbiosis can also lead to infection by opportunistic pathogens such as *Escherichia coli* and *Clostridioides difficile* (Theriot *et al*., 2014). Therefore, the precise manipulation of the human microbiome to eradicate pathogens or restore eubiosis – that is, balanced microbiome – in diseases associated with dysbiosis and also maintain this balance is of great interest.

Microbiome manipulation

Current modulation strategies

We have only recently started to appreciate the role of microbiome in our body's maintenance and development. Yet, their manipulation started much earlier and dates back to 4th-century China when faecal transplantation was used to treat severe food poisoning and diarrhoea (Zhang et al., 2012). The discovery of probiotics also goes back to the 19th-century when *Lactobacillus bulgaricus* and *Lactobacillus acidophilus* were introduced for their possible benefits to human health (Podolsky, 2012). More recent examples of intervention methods, however, are prebiotics – dietary compounds that selectively promote the growth of beneficial gut bacteria (Collins *et al*., 2018). Inulin, a dietary fibre, improves insulin sensitivity in obese adults with distinct effects on the gut microbiome such as increased *Actinobacteria* and decreased *Clostridiales* (Chambers *et al*., 2019). One more example of this is glycan – a dietary compounds – that is consumed by *Bacteroides*

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thetaiotaomicron, a gut symbiont, with the potential to shape the gut microbiome (Marcobal *et al*., 2017). Likewise, antibiotics have shown some promise in attenuating the development of diseases such as IBD (Prantera *et al*., 2006) and CRC (Zackular et al., 2016) by depleting the gut microbiome.

Although these strategies have shown promising results through improving microbiome structure, regulating the immune system, suppressing pathogens, decreasing toxin levels and attenuating tumour development, there are also some concerns about their efficacy and safety. For example, faecal transplant, which is highly effective against recurrent *C. difficile* infection and shows promise in treating intestinal graft-versus-host disease (GvHD) (Lier *et al*., 2020), can also transfer harmful pathogens such as extended-spectrum beta-lactamase (ESBL) producing *E. coli* (DeFilipp *et al*., 2019) or induce obesity (Alang and Kelly, 2015). The main concern about probiotics is their efficacy as they often fail to induce a long-lasting impact on gut community structure and are highly individualised (Zmora *et al*., 2018). Most importantly, these methods have low specificity that can lead to large-scale changes in the body instead of specific eradication of disease-relevant taxa.

Can we use phages?

Phages – as microbiome modulators – can address multiple issues mentioned earlier, including specificity and safety. Phages are bacteria's natural enemies and have evolved a wide array of highly diverse antibacterial strategies over billions years of co-evolutionary struggle with their bacterial hosts. In addition, they have a high level of host specificity – at strain level for some phages – thus are safe to human cells (Khan Mirzaei and Maurice, 2017; Dąbrowska and Abedon, 2019). Phages have been continually used to treat infections in Eastern Europe since the 1930s, and there has been growing worldwide acceptance of their potential as antimicrobials in recent years (Dąbrowska and Abedon, 2019). In the Western world, several successful applications of phages against multi-resistant bacteria on single patients have recently been reported (Chan *et al*., 2018; Dedrick *et al*., 2019). They also have recently shown promising regulatory effect on gut bacteria (Reyes *et al*., 2013; Duan *et al*., 2019; Khan Mirzaei, khan *et al*., 2020). Yet, there are some limitations to using phages that need to be addressed before their application becomes a reality.

Drawbacks and the help of emerging technology

Complexity of the infection network

Current understanding of phages–bacteria interactions is mostly based on reductionist studies of single-phage-single-host dynamics. Even the recent metagenomic studies have only provided little insights into the mechanics of phages–bacteria interactions in complex ecosystems such as the human gut while they mostly identified their community composition (Deng *et al*., 2014; Džunková *et al*., 2019; de Jonge et al., 2020). These studies all lack details on phages–bacteria interaction patterns and have failed to unveil the individual infection network for phages in that complex community (Weitz *et al*., 2013; Džunková *et al*., 2019; Khan Mirzaei, khan *et al*., 2020) – which is information that will be necessary for phage-based manipulations. Culture-independent methods such as viral-tagging (VT), which uses fluorescence-activated cell sorting, when combined with single-cell metagenomics, can precisely predict unique host–phage pairings in both the marine (Deng *et al*., 2014) and human (Džunková *et al*., 2019) ecosystem. For example, a recent study revealed over 360 novel phages–bacteria interactions within the faecal samples from 11 healthy volunteers using VT, which could not be detected with culture-based methods (Džunková *et al*., 2019).

Not at full capacity

Most of the progress on phage therapy has been relying on a very limited number of phage isolates, which slows down the global acceptance of phages as valid therapeutics. From the estimated 10^{31} phages on earth, only $< 10^4$ are isolated and sequenced (NCBI, June 2019) – mostly those that could be cultured. This means that we are mostly counting on phages with very similar infection strategies for treatment. Yet, with the recent advances in bacterial culturomics, a groundbreaking cultivation method that uses multiple growth conditions and multi-omics, it has become possible to culture bacteria that were previously believed to be unculturable (Lagier *et al*., 2016). In light of these advancements, we propose VT-culturomics that combine single-cell VT with culturomics enabling high-throughput screening, isolation and the reproduction of unknown phages while identifying their phenotypes and characterizing their interactions with the known bacterial host (Deng *et al*., 2014; Lagier *et al*., 2016). In addition, VT-culturomics uses artificial intelligence (AI) for data evaluation, robotic control and state-of-the-art prediction of the phages kinetics. This allows the isolation of hundreds of new phages in a single run (Fig. 1), leading to the identification of unknown phage antibacterial strategies. Altogether, these will expand our arsenal against any target bacteria that cause multi-resistant infections or are associated with a chronic disease. It also provides exciting new perspectives on phages–bacteria relationships.

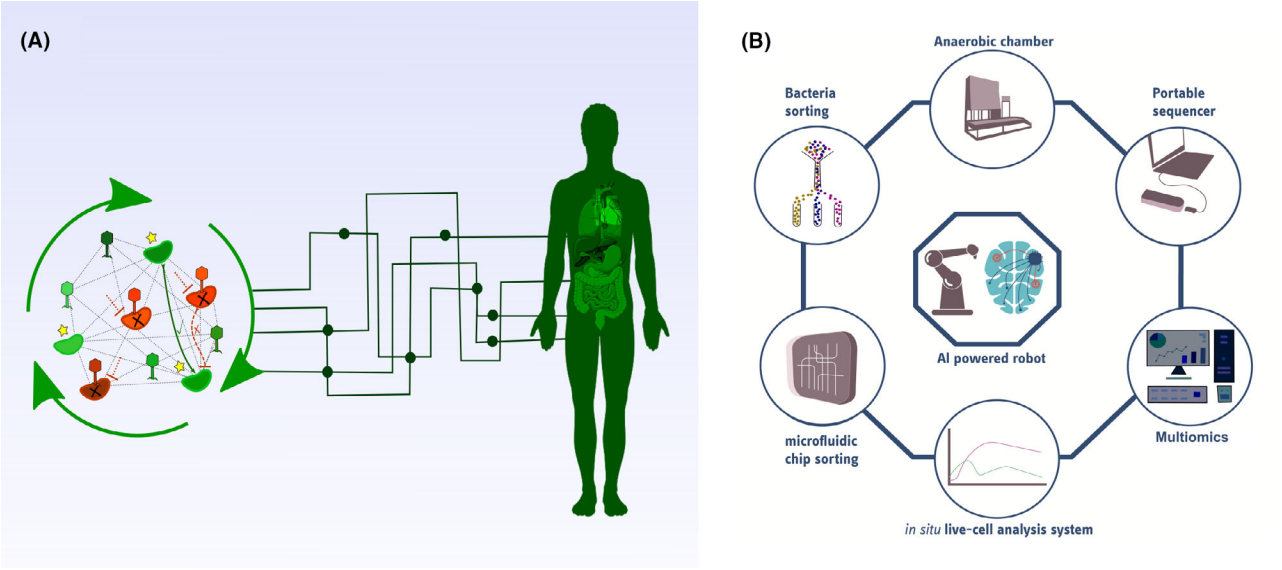

Fig. 1. Strategies towards establishing a sustainable microbiome. A. synthesized phage-based modulators selectively manipulate the structure and function of the human microbiome. X: selectively eliminates bacterial strains, *adding fitness genes to bacteria, x: blocking the spread of virulent genes, ↜: phage-mediated gene transfer. B. VT-culturomics pipeline which uses the artificial intelligence (AI) for data evaluation and robotic controlling, single-cell VT combined with multi-omics and live-cell analysis for characterizing the complex microbial community.

We can even turn this duo into a trio, by integrating advanced synthetic-biology techniques into the VT-culturomics pipeline to further improve its function. Using these methods, we can synthesise not-yet-cultivated phages identified in VT-metagenomic without the presence of a suitable host – in a cell-free system or via yeast-based gap-repair synthetic platforms (Gibson *et al.*, 2008; Yim *et al.*, 2019) – and engineer them to (i) enhance their antibacterial activity by suppressing bacterial SOS response, (ii) shift host range through modifying phages' receptor binding proteins (RBP) or adding multiple RBPs (Ando *et al.*, 2015; Dunne *et al.*, 2019), (iii) reduce their immunogenicity by altering immunogenic phage structural proteins (Hodyra-Stefaniak et al., 2019) or (iv) deal with the bacterial resistance mechanisms by expressing a biofilm-degrading enzyme or anti-CRISPR protein (Lu and Collins, 2007; Bondy-Denomy *et al.*, 2013). Ultimately, these advances allow for (i) molecular characterization of the underlying mechanisms of phages–bacteria interactions on the strain-level and (ii) will pave the way for large-scale perturbations of community structure and function at an unprecedented scale on the community-level.

An alternative approach for manipulating microbial communities also involves phages – those integrated into bacterial genomes called prophages and described as 'molecular time bombs' (Paul, 2008). The targeted induction of these prophages in specific bacteria by dietary compounds (Oh *et al.*, 2019; Boling *et al.*, 2020) or antibiotics (Modi *et al.*, 2013) results in the removal of these bacteria and allows outcompeted taxa to grow, restoring balance in dysbiose communities.

Sustainable microbiome

Even though the precise manipulation of the human microbiome that can restore eubiosis in diseases associated with dysbiosis is the essential first step, the new balance should be maintained to produce a long-term effect on the host. Therefore, we suggest the sustainable microbiome – a robust and resilient microbial community that remains diverse and balanced in response to the internal or external dynamic changes – as a healthy microbiome.

We expect that VT-culturomics in combination with synthetic-biology techniques will provide an array of highly specific, superefficient and riskless tools for precise manipulation of every microbial community on earth, including the human microbiome. Using these tools, we can (i) selectively eliminate any pathogenic bacteria, (ii) transfer beneficial genes to a specific bacterial host and promote its growth, (iii) insert new functions, beneficial to the human host, to existing taxa and (iv) block the spread of virulence genes in microbial communities, thus moving from engineering individual organisms to entire ecosystems (Fig. 1). The last three steps can be achieved using native or engineered phages as delivery systems for transferring genes or genetic circuits. The metabolic function of the microbial communities can also be altered by regulating the gene expression in bacteria through phage-

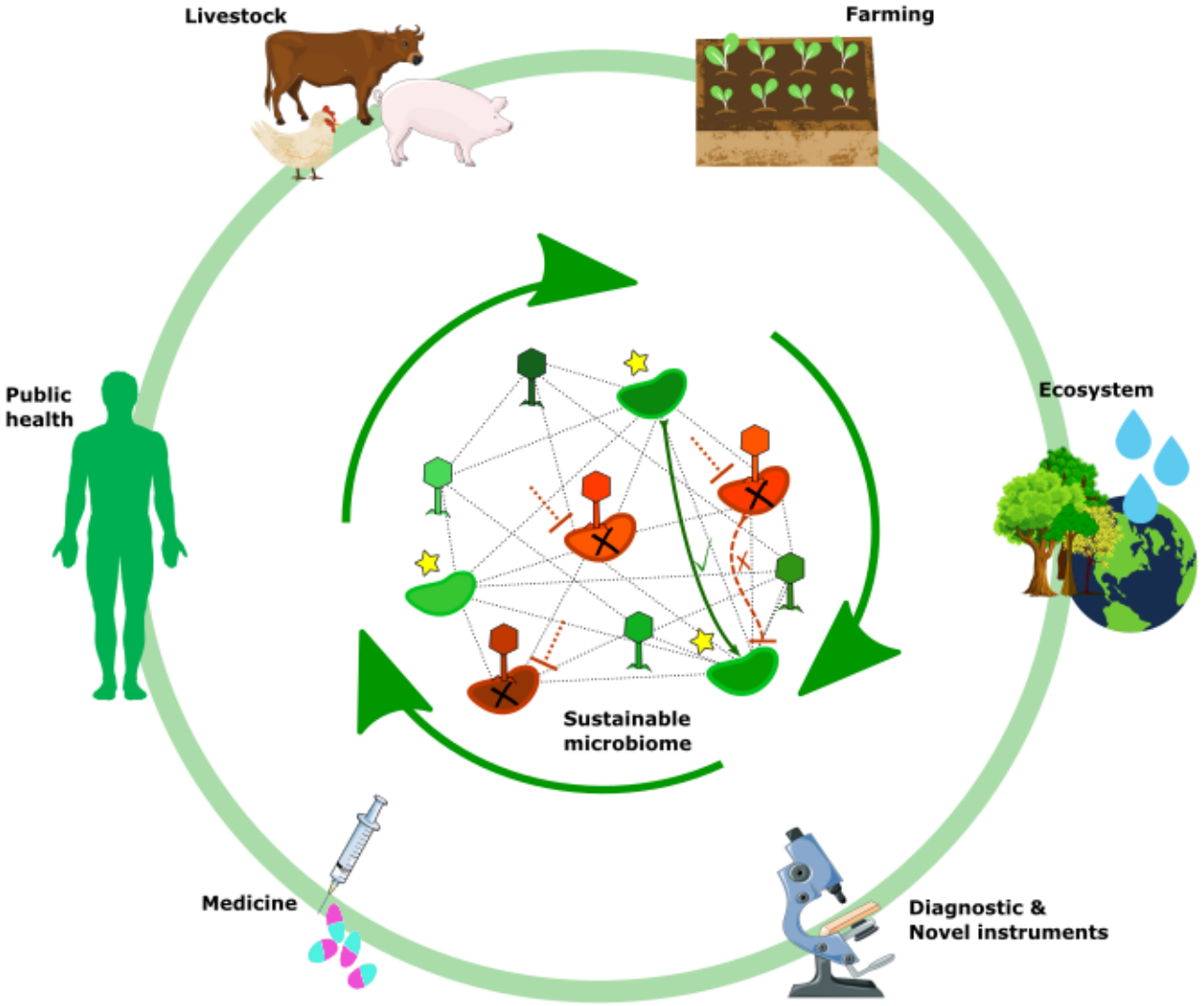

Fig. 2. Extended application of sustainable microbiome. X: selectively eliminates bacterial strains,: adding fitness genes to bacteria, x: blocking the spread of virulent genes, ↙: phage-mediated gene transfer.

mediated integration of exogenous genes into their genome. This will change the communities at the population level by altering the abundance of specific strains or introducing competitive or cooperative interactions.

To this end, we can enhance, exclude or modify the structure and function of the microbiome towards establishing a self-sustained community by (i) avoiding large-scale non-specific changes due to the high specificity of phages, (ii) not adding foreign bacteria which may not survive in the new ecosystem, but instead promoting the existing outcompeted taxa in the community and (iii) personalizing the treatment – through pre-characterization of the commensal microbial and viral/phage community, and the creation of specific phage-based modulators (Fig. 1).

In addition to their obvious potential in treating dysbiosis-associated disease in humans, these strategies can be used to edit microbial communities in other systems. For example, to engineer the cow's gut microbiome to reduce methane production or accelerate nutrient uptake by manipulating the plant microbiome. In the natural environment, the introduction of biodegradation pathways by synthetic phages can potentially remove soil contamination. In the built environment, such as public transportation, hospitals and schools, creating a sustainable microbiome can boost human health by increasing microbial diversity while eliminating pathogens (Fig. 2).

Concluding remarks

In the last two decades, microbiome research has changed our understanding of these microbial communities' structure, function and regulatory roles in different

ecosystems, including the human body. In addition, the amenability of these communities is opening up a new area of research that aims to treat dysbiosis-associated disease or conditions by precise editing of the microbiome. Yet, we have a long way to go before this becomes a reality. We envision that recent advances in single-cell technologies, culturomics and synthetic biology offer exciting opportunities by (i) unveiling the detailed interactions between different members of these communities, specifically phages and bacteria and their underlying mechanisms, and (ii) providing meticulous modulatory tools for precise editing of these complex microbial communities towards creating a sustainable microbiome.

We expect that the current strict regulations on genetically modified organisms (GMOs) will be relaxed in the near future, boosting the application of phage-based editing tools in healthcare, improve health and save more human lives.

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Conflict of interest

None declared.

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