Pharmacology in the age of the Holobiont

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Highlights

- Pharmacomicrobiomics - new frontier in pharmacology
- Holobiont – the physiological unit of host and microbes as a drug target
- Drugs and drug targets are re-defined in the context of the holobiont
- New holistic approach in drug research using holobiont animal models
- Exciting new prospects for microbiota-based personalised medicine

Glossary:

Holobiont - Supra-organism of host and its microbes.

Pharmacogenomics – Field of research which combines pharmacology and genomics to study how the genetic make-up of an individual modulates its response to therapeutic drugs [1].

Pharmacometabolomics – Field of research which combines pharmacology and metabolomics to study how the metabolome shaped by environment and genetics influence the response of an individual to therapeutic drugs [2].

Xenobiotics – An exogenously produced chemical compound found inside an organism. This broad term encompasses therapeutic drugs and dietary metabolites.

Abstract

Despite the widely acknowledged fact that the microbiota regulates many aspects of human health, the dynamics and factors that govern these interactions remain mostly unknown. Pharmacomicrobiomics is a new research frontier in pharmacology that studies the interaction between drugs and the microbiota. This discipline, by including the microbiota as a key regulator of host health, calls for a redefinition of what constitutes a drug target and ultimately what is a drug or drug therapy. This is supported by recent evidence showing that host physiology can no longer be studied in separation from its microbial ecology and the environmental factors that shape it, as the combination of these elements forms the physiological unit of study – the holobiont. Here we discuss both the novel challenges and untapped opportunities that this new framework creates. On one hand, a more complete understanding of the physiology of the host imposes the development/adaptation of new animal models to address these interactions. In particular, we focus on the advantages and disadvantages of *C. elegans* as a host organism. On the other hand – a complete understanding of the effects of the microbiota and xenobiotics (e.g. drugs and dietary metabolites) on host health opens new prospects for personalized therapy.

Introduction

Pharmacology has come a long way since the inception of “magic bullet” drugs at the beginning of the 20th century. Intelligent drug design still holds the promise of devising new compounds that specifically affect a disease target yet are harmless to the functioning of the entire organism. The lack of success in achieving such gold standards, associated with high failure of new drugs in clinical trials has led to an increasing interest for drug repurposing [3,4]. Developments in molecular and cellular biology have revealed a number of new drug targets and factors that may affect efficacy and/or side-effects. As a consequence, new branches of pharmacology emerged, like pharmacogenomics and pharmacometabolomics, which aim at understanding how the genetics and the metabolism of an individual can modulate drug efficacy.

Despite great advances in pharmacology, one factor often disregarded in developing personalised medicine is the role of the gut microbiota in the modulation of drug effects. The microbes inhabiting the gut of an organism have a dramatic role in regulating host health [5] and are the target of an unexpected high number of drugs originally designed at targeting host processes [6]. As a consequence, the recent revolution in microbiome research established a new frontier in pharmacology. Pharmacomicrobiomics aims at tackling the interplay between drugs, microbiota ecology, environmental pressures and host genotype [7-9]. The full scope of these interactions may only be understood using holistic approaches, thus requiring a shift in our understanding of the human body. Together with their microbiota, animals form the holobiont [10] – a single physiological entity with a combined metagenome, and metabolism that is shaped by its environment [11,12] (**Figure 1a**). Therefore, redefining the holobiont as a target of drug therapy rather than the host only, may also call for a re-evaluation of what therapy is, to include compounds that directly or indirectly affect drug efficacy through an indirect impact on the microbiota.

The holobiont exists in a constant flux of dietary metabolites and drugs, which are taken from the environment and circulated between the host and its microbes. In terms of their biological effect, these compounds can be foodstuff, inert, regulatory or toxic molecules. Often there is a shared chemical space between the host and the microbiota – co-metabolism. In this context, microbes and host share identical or similar metabolic pathways allowing them to exchange and utilize metabolites indiscriminately within the holobiont. In other cases, microbes and host complement each other metabolically - where the products of the metabolic machinery of one organism can be utilized by the other (**Figure 1b**). For example, microbes utilize dietary fibre as a nutrient source [13,14], which is mostly inert matter for the human host, and the resulting short-chain fatty acids positively regulate host physiology. However, these concepts are relative and ultimately depend on the specific microbes and the host cell type [15,16]. Ultimately, the phenotype of the holobiont is shaped by the metabonome [17] shared between the microbiota and the host that operates in a complex chemical feedback loop (**Figure 1c**). Therefore, transient environmental factors like diet and drugs may alter the function of the microbiota leading to the production of metabolites that can act as adjuvants or impair drug action on host metabolism [7,18] or alternatively, may impact the direct transformative role of the microbiota in chemically modifying drugs [19]. In such scenarios causality in drug action may easily be lost leading to unanticipated and poorly understood mechanistic effects. This further highlights the increasing need to adopt such an integrative view of physiology and pharmacology for drug research and development.

Altogether, the recent developments in pharmacology stress the uniqueness of each

patient as a result of its genome, microbiome and intricate relationship with the environment. Such complexity underscores the challenges ahead for precise personalized medicine but also reveals a myriad of untapped opportunities for therapy [20]. So, what is needed to bring pharmacology into the age of microbiome? How can we use animal models to feasibly, accurately and predictively tackle the ever-increasing complexity of drug action and ultimately draw insights which will lead to the improvement of therapies? In this short review, we highlight some of the key ideas at the intersection of microbiome and pharmacology research and provide hints for potential future trends in these fields.

Targeting the structure of the microbiome for health

The human microbiota is formed of trillions of bacterial cells, viruses and fungi that inhabit the various anatomical regions of the human body [21] and there is a general consensus that it plays a crucial role in host health [11]. The in-depth analysis of the structure and the composition of our microbiota was enabled by the shift to a culture-independent approach using 16S rRNA and shotgun metagenomic sequencing [22]. Major population studies have revealed that the microbiome is remarkably stable in healthy adults [23] and vastly rich in metabolic functions [24,25]. It contains 100x more genes than the human genome, with 50% of its content not fully annotated and likely to contribute greatly to the extended metabolic capacity of the holobiont [11,12]. Each persons' microbiome is unique and exhibits wide inter-personal and intra-personal variation, with distinct bacterial communities inhabiting different anatomical regions [21]. Given the complexity of this ecological niche and its quickly adaptable nature, it is not only difficult to define the core composition of a healthy microbiota but also to fully capture and define the mechanisms that govern the transitions between healthy and unhealthy states [26]. Additionally, since most human microbiome studies are retrospective and our control of the variables is governed by ethical or technical limitations, unknown factors may drive structural changes in microbiome communities and thus unknowingly define the holobiont phenotype. This was illustrated by the driving effects of the antidiabetic drug metformin on the structure of the microbiota in a human type-2-diabetes (T2D) study [27]. Two gut metagenomics studies of T2D patients that did not take drug treatment as a key confounding variable yielded conflicting conclusions regarding the nature of the disease-associated gut microbial dysbiosis [28,29]. Disentangling drug effects allowed for the identification of a unified microbiome signature induced by T2D and revealed a positive impact of metformin on the microbiota contributing partly to its therapeutic effects [27].

Therefore, the lack of a complete mechanistic understanding of such microbiome shifts is troubling, because the adaptable nature of the microbiome also makes the holobiont phenotype a moving target for therapy. One potential solution is the stratification of microbial communities into functional states called enterotypes [30], as major indicators of structural trends. Such strategies may ultimately become useful in the clinical setting, providing an opportunity for the microbiome's systematic investigation, classification and potential targeted alteration.

Harnessing the power of the microbiome – Drugs

The ecological niche of the human microbiota can be shaped and perturbed by multiple factors [11]. Firstly, it is defined by the physical properties of the anatomical location [21], the genotype [31] and the age of the host [32,33]. Then, multiple transient environmental factors, over the lifetime of the host, alter or perturb its microbiota ecology,

namely lifestyle [34], infections [35], hygiene [36], diet [15,37-39] and exposure to xenobiotics [7,16,40] (**Figure 1a**). Research suggests that environmental factors are the major drivers in shaping gut microbial communities, overruling host genotype [37,41] [25,42]. Remarkably, *in vitro* assays show that 24% of over a 1000 of host-targeted drugs impaired the growth of at least 1 out of 40 representative human gut bacterial strains [6] supporting a key role for drugs modulating the microbiota. The antidiabetic drug metformin, also identified in this study, portraits part of the complexity involved in holobiont-drug interactions. Studies show that the drug has distinct effects on both host and microbial cells but the combined interactions of drug-host plus drug-microbe determine the holobiont phenotype. In fact, metformin was shown to induce important structural [27,43], transcriptional [43] and metabolic changes [44] in the microbiota, but also in the metabolism of the host [1], ultimately contributing to its efficacy in the regulation of host glucose homeostasis [43] and lifespan [44]. Additionally, the microbiota can indirectly modulate drug effects by producing compounds that can compete directly with drugs (e.g. acetaminophen and p-cresol) for their modification by enzymes of the host [7].

Microbes can also directly alter drug pharmacokinetics by activating, reactivating and inactivating drugs. Currently, around 60 drugs are known to undergo several types of chemical modification by the gut microbiota [7,9]. This forms an important component of the first-pass drug metabolism together with the intestinal and hepatic enzymes of the host. Prodrugs, like protonsil, can be activated by microbial azoreductases cleaving their azo bond – a feature that was accounted for during drug design. Conversely and unexpectedly, up to 50% of cardiac glycoside digoxin can be rendered inactive through biochemical reduction by *Eggerthela lenta* [7]. Two additional types of co-metabolism in the context of drugs are elegantly illustrated by the anticancer drugs irinotecan [45] and fluoropyrimidines [46]. The anticancer agent irinotecan is inactivated by hepatic glucuronidation and then reactivated by bacterial b-glucuronidases after biliary secretion. This also demonstrates that oral intake is often not a prerequisite for drug-microbe interactions due to biliary and epithelial excretion. Fluoropyrimidine pro-drug efficacy, on the other hand, depends on the relative contribution of the metabolic ribonucleotide pathways of the host and its microbes to biochemically activate these compounds and ultimately target host cellular division [46,47]. Further, metabolites and molecular cues from *E. coli* and *F. nucleatum*, respectively, modulate the activation of cellular autophagy and thus the effect of fluoropyrimidines [46,48] on cancer progression.

Altogether, these studies illustrate two important principles. One, that drugs can be used to selectively edit the microbiota by suppressing or increasing the abundance of specific bacterial strains involved in regulating host phenotypes (e.g metformin [27,43]). Two, dietary cues or drug-drug combinations could be used in tandem as tools for modulating the bi-directional metabolic/signalling communication that exists between host and microbe in the context of drug efficacy. Overall, given the responsive nature and the unique role of specific microbes in maintaining different aspects of health [49], the microbiota provides exciting prospects to be explored either as a therapy itself or as a target of drugs [44,50] for health benefits.

Harnessing the power of the microbiome – Diet

Given the microbiota's sensitivity to a diverse range of xenobiotics apart from host-targeted drugs and antibiotics, personalised nutrition has emerged as a new therapeutic paradigm. Diet is therefore being viewed as an instrument to shape the microbiota and in

turn, the holobiont phenotype [8,18,38,41]. Such modification may involve the use of prebiotics (e.g dietary fiber, inuli type-fructans), which shape the microbiota by promoting growth of specific bacterial phyla [15] and provide general benefits to the host [13,14,51]. However, little is known about the mechanisms by which nutrients and/or metabolites from our diets influence the holobiont. Human diets are naturally complex, composed of an undefined mishmash of macro and micronutrients. Thus, understanding the effect of a single or a combination of nutrients/metabolites on the holobiont phenotype is an essential but daunting task. The metabolism of L-carnitine, an important dietary component of red meat, is an example of complementary metabolism for dietary cues between the microbiota and host, with implications for the health of the holobiont. Bacteria convert L-carnitine to trimethylamine which is further metabolised by the liver of the host to trimethylamine N-oxide, a product that promotes atherosclerosis [52]. Another example is provided by the metabolism of tryptophan, an aromatic amino acid. Tryptophan, can either be converted by gut bacteria to produce indoles that improve healthspan [53] or alternatively, the uremic toxin indoxyl sulphate which can cause chronic kidney disease [54] (**Figure 1b**). Therefore, to better understand the effects of nutrition and define dietary approaches as therapy, we first need to develop comprehensive and integrative methods that allow the causal investigation of the complex interactions between host, microbes and nutrition in the regulation of the phenotype and health of the holobiont.

Experimental holobiont models

Microbiota research is moving away from descriptive correlative studies to in-depth mechanistic analysis of both its function and therapeutic implications. The complexity of the holobiont is intimidating due to a vast number of variables that shape its phenotype. Some of these challenges can be met by experimental animal models, but they need to fulfil a number of requirements to provide new insights on the biology of the holobiont [12,55] (**Figure 2a**). Bacterial community ecology remains challenging to investigate, model and engineer [56,57]. Microbial ecosystems can be studied through phenotype screening of cultivable microbes [58]. This allows the characterization of the microbes in this community based on general physiological properties, sensitivity to drugs [6] and nutritional preferences [59]. Additionally, it provides a platform for the detailed modelling of shared metabolism between bacterial members [60]. Semi-artificial cellular platforms created by gut-on-chip organoid models [61] may be used to reveal the biological principles in the interaction between complex eukaryotic cellular environments and prokaryotic ones. The simplest whole-organism model with a microbiota of an intermediate complexity is *C. elegans* [62,63]. Easy to handle, cost-effective and scalable, it allows the systematic high-throughput investigation of the effects of the microbiota on a host's phenotype [64]. *Drosophila* is unique in its defined holidic nutritional media [65] and a simple native microbiota comprising of approximately 20 bacterial species, which allow the study of nutrition-microbiota-host interactions in diverse evolutionarily conserved host traits [12,66-68]. Zebrafish and *Nothobranchius Furzeri* are emerging as powerful models to study microbiota effects in vertebrates. For example, the transparency of zebrafish enables the live and real-time investigation of gut colonisation dynamics using fluorescently-labelled bacterial strains [69]. *N. Furzeri* has become instrumental for the study of the role of the microbiota in organismal ageing due to its relatively short lifespan for a vertebrate animal [70]. The common mammalian model of choice are mice [37,71], whose microbiota has been standardised [72] and can also be humanised [73], even though it lacks the scalability

offered by lower animal and non-animal models. However, the closest to humans but rarely used model is the pig, which can also receive and maintain human microbiota [74]. Overall, all models provide unique benefits but also distinctive drawbacks and the researcher's choice should ultimately depend on the scope of the study and the nature of the problem that is being investigated.

C. elegans is the simplest complete holobiont model organism, providing proxy readouts for most aspects of host-microbiota interactions. It is uniquely scalable and can feasibly address the impact of environmental cues (e.g. drug, nutrition), host genetics and microbial genetics in different aspects of holobiont physiology (e.g development, reproduction, metabolism, ageing) (**Figure 2b**). *C. elegans* has a short generation time of 3 days and a lifespan of 3 weeks with isogenic, transgenic and gnotobiotic animals easily maintained. It is arguably the best genetic multicellular model with powerful forward and reverse genetics screen tools, in addition to transgenesis and CRISPr/Cas9 gene editing tools for the identification of genes and their function in the regulation of holobiont phenotypes. Its native microbiome is intermediately complex [62,63], but worms can also grow in lab conditions on a single or a community of bacterial strains which act as both nutritional source and gut commensals [64]. Unfortunately, worms are normally cultured at ambient levels of oxygen, which imposes a restriction on the study of microbes that are mainly aerobic. However, it remains to be investigated whether the worm gut is microaerophilic or anaerobic, with the potential capacity to maintain strict or facultative anaerobes. Given the critical relationship between gut microbes and host immunity, the lack of an adaptive immune system is a drawback of this holobiont system, which can impose limits on the translatability of findings to humans.

The greatest advantage of *C. elegans* in comparison to other models is its high-throughput screening capacity, not lagging far behind classical high-throughput microbiology approaches [16,46,75]. For example, bacterial knockout *E. coli* libraries [76] can be used together with wildtype worms [77,78] or possibly in tandem with RNAi libraries [79], or mutation genetic libraries [80] for the study of the interaction between microbial genetics and host genetics. The worm can also be used to assess the influence of bacterial genetics in regulating drug efficacy on host physiology, such as the role of anticancer drugs [46,47,81] or metabolic drugs such as metformin [44]. In addition, GFP transcriptional reporter *E. coli* libraries [82] could be used to study the influence of host genetics on the regulation of bacterial gene expression within a community of microbes during the gut colonization – all enabled by the transparency of host's body. With more widespread adaptation, the simplicity of this model will yield unique advantages in resolving complex problems. It may, for example, enable early high-throughput *in vivo* testing in drug development pipelines and provide an excellent platform for the discovery of bacterial genes and/or processes regulating evolutionary conserved mechanisms in the holobiont.

Conclusions

The widespread role of the microbiota in regulating the health and wellbeing of the holobiont provides exciting avenues for therapy but our current understanding of the complex interactions between host, microbiota and its environment is still in its infancy. Therefore, research focusing on the microbiota has the potential to drastically change pharmacology and our approach to human therapies. As the most recent iteration of personalised medicine, pharmacomicrobiomics highlights the necessity to investigate different metabolic parameters of the microbiota in order to avoid spurious drug

interactions and allow the development of more robust therapies. Its implications extend far beyond prevention of undesired interactions, with the possible targeted use of xenobiotics to alter microbiota for host health benefits. However, the ability to temper with the microbiota for therapeutic purposes requires a more systematic and mechanistic investigation of the holobiont, to define the functional states of the microbiota, the factors that shape it and their biological effects on the host. Such approaches may not be able to rely on the known sets of scaffolds and drug-likeness principles of compounds aimed at the host. New guidelines may result from pharmacomicrobiomic research as we build on the solid mechanistic understanding of basic processes and extrapolate them to more complex settings. Scalable holobiont models, in particular *C. elegans*, already embody such philosophy and allow the detailed and controlled investigation of multiple factors that shape the physiology of the holobiont. Ultimately, discoveries made in animal models should lead to therapeutic predictions using metabolic models [57] (**Figure 2c**). For example, important progress has already been made in establishing the necessary computational tools [83] and reconstructing metabolic models of bacterial strains from the human microbiota [84]. There is also a community effort to develop the first metabolic model of a multicellular organism – *C. elegans*, which will incorporate the metabolic contribution of gut commensals [85]. In the future, such approaches might provide valuable insights when transitioning from animal testing to human trials. These are the very first steps in the field of pharmacomicrobiomics that will likely introduce new therapies, but more than that, it will without a doubt bring a more complete understanding of our health.

Figure captions:

Figure 1: (a) Holobiont – a holistic framework in pharmacology which combines host, microbiota and environmental interactions. Traditional pharmacology concentrates primarily on the drug effects on host physiology with respect to the variation in host genome (pharmacogenomics) and metabolome (pharmacometabolomics). However, host development, health and homeostasis intricately depend on microbial ecology, which itself can become a target of therapy (pharmacomicrobiomics). This reveals both exciting opportunities and daunting challenges, as the microbiota can be easily moulded by multiple environmental factors. (b) The holobiont exists in a flux of xenobiotics (e.g. dietary metabolites and drugs) which may have differing effects on host and bacterial cells. Bacteria may also transform these compounds (green arrows) thus contributing to their indirect effects. (c) The Holobiont results from the combination of the metagenome and metabonome shared by the host and its microbiota and shaped by a complex feedback loop resulting from their interaction.

Figure 2: (a) General comparison of experimental holobiont animal models and humans in terms of host, microbiota and environmental properties. (b) *C. elegans* – scalable holobiont model, which can be used in high-throughput screens to unravel the complexity of host, microbiota and environment interactions. (c) Findings in experimental setting may be integrated using *C. elegans* and *E. coli* metabolic model and further extrapolated to humans.

Acknowledgements
F.C. acknowledges funding from the Wellcome Trust/Royal Society (Sir Henry Dale

Fellowship-102532/Z/12/Z) and P.N. from the Wellcome Trust. We would like to thank Dr. Kevin Bryson for critical reading of the manuscript. We apologize for the exclusion of some of the research findings and less commonly used holobiont models due to the limited scope of this review.

Recommended reading (* of special interest, ** of outstanding interest):

- ** Scott 2017 & Garcia-gonzalez 2017 – First studies of the mechanisms by which commensal microbes alter the efficacy of anticancer fluoropyrimidine drugs using a *C. elegans* holobiont model. Development of 3-way host-microbe-drugs high-throughput screens.
- ** Zeevi 2015 - In this study patient glycemic response to diet was successfully predicted using their microbiota composition and other health metrics. These predictions were then used to correspondingly change patient diet. This highlights the use of personalized nutrition as a therapeutic strategy.
- ** Leulier 2017 – This paper highlights the need for an integrative physiology framework which incorporates both microbiota and diet as important factors shaping the host phenotype.
- ** Maier 2018 – In this study, 40 gut microbiota representative bacterial strains were challenged with over 1000 host-targeted drugs. This study highlights the extensive impact drugs can have on the microbiota which can be responsible for drug associated side-effects and/or health benefits.
- * Cabreiro 2013 – First study that shows that the antidiabetic drug metformin induces changes both in microbial metabolism and the host, which ultimately contribute to the phenotype of the host.
- * Stappenbeck 2016 – This paper highlights the challenges of performing microbiota research using traditional animal models.
- * Spanogiannopoulos 2016 – This paper presents a detailed overview of drug/xenobiotic-microbiota interactions.

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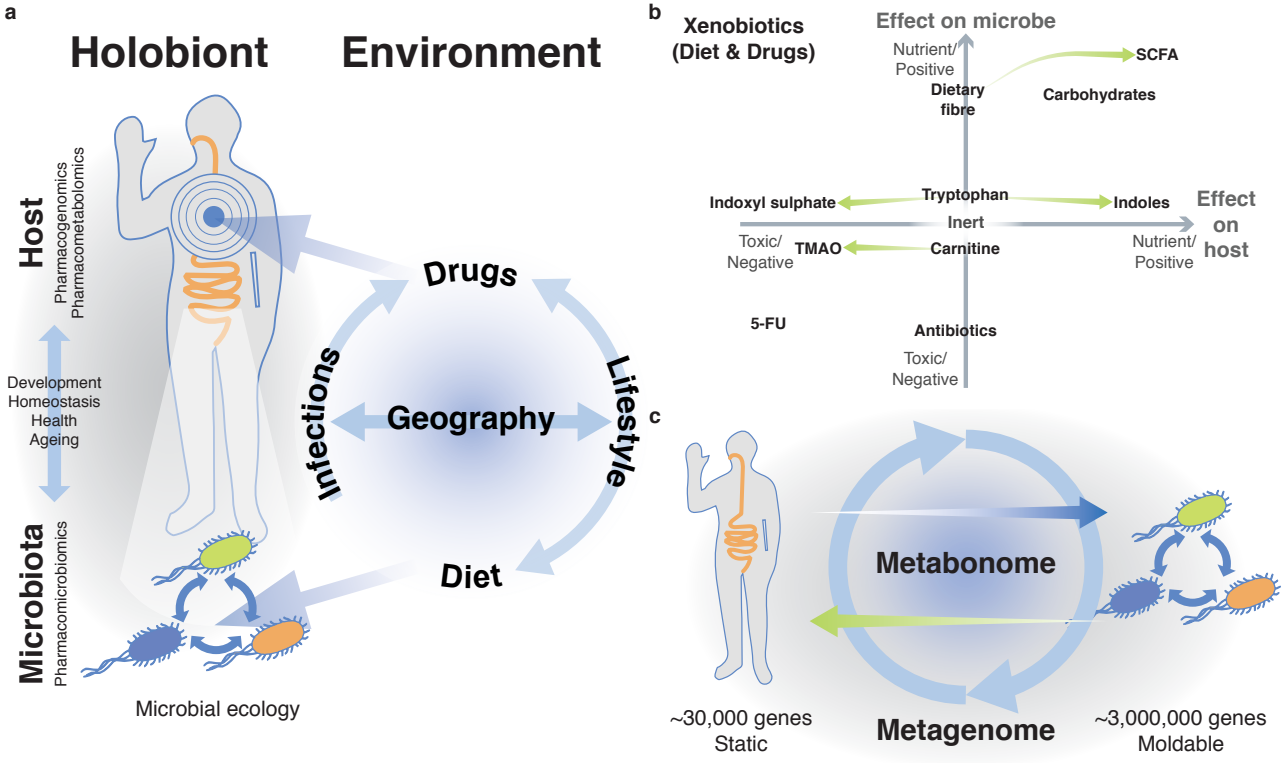
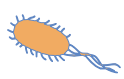
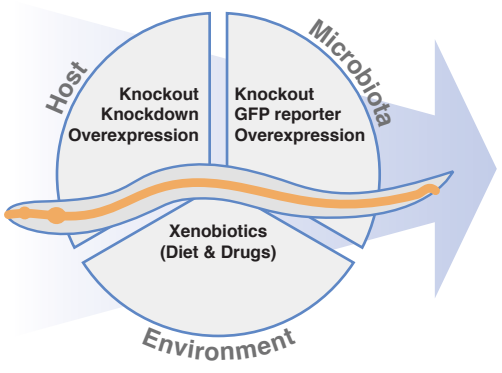

a
Holobiont
Environment
Host
Pharmacogenomics
Pharmacometabolomics
Development
Homeostasis
Health
Ageing
Microbiota
Pharmacomicrobiomics
Microbial ecology
Drugs
Infections
Geography
Lifestyle
Diet
b
Xenobiotics
(Diet & Drugs)
Effect on microbe
Nutrient/
Positive
SCFA
Dietary
fibre
Carbohydrates
Indoxyl sulphate
Tryptophan
Indoles
Inert
Effect
on
host
Toxic/
Negative
TMAO
Carnitine
Nutrient/
Positive
5-FU
Antibiotics
Toxic/
Negative
c
Metabonome
Metagenome
~30,000 genes
Static
~3,000,000 genes
Moldable

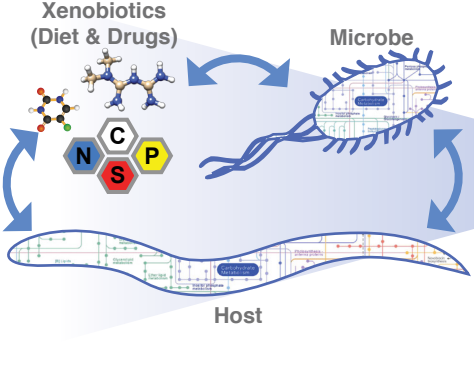
a

		Bacterial	"Gut-on-chip" organoid	Worm	Fly	Zebrafish	Rodent	Piglets	Human
									
Complexity									
Type	Host	–	Artificial	Invertebrate	Invertebrate	Vertebrate	Mammal	Mammal	Mammal
Phenotype readout	Host	–	–	Simple	Simple	Complex	Complex	Complex	Complex
Transparent	Host	–	Yes	Yes	No	Yes	No	No	No
Genetic libraries available	Host	–	Yes	Yes	Yes	Yes	Yes	No	No
Transgenesis/knockouts	Host	–	Very easy	Easy	Easy	Medium	Medium	Hard	No
Isogenic	Host	–	Yes	Yes	Yes	Yes	Yes	No	Yes
Generation time	Host	–	Hours	Days	Days	Weeks	Weeks	Months	Years
Lifespan	Host	–	–	3 weeks	2 months	4 years	3 years	10–15 years	Decades
Native microbiota complexity	Microbiota	–	–	Intermediate	Simple	Complex	Human–like	Human–like	Human
Gnotobiotic maintenance	Microbiota	–	–	Easy	Medium	Hard	Hard	Hard	No
Single bacterial strain can be used	Microbiota	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
Bacterial libraries can be screened	Microbiota	Yes	Yes	Yes	Yes	Unfeasible	Unfeasible	Unfeasible	No
Aerobic/Anaerobic	Microbiota	Both	Both	Yes/No	Both	Both	Both	Both	Both
Availability of mock communities	Microbiota	–	–	Yes	Yes	No	Yes	No	No
Cost of maintenance	Environment	Low	Low	Low	Low	Medium	High	Very high	–
Special facilities needed	Environment	No	No	No	No	Yes	Yes	Yes	–
Standardised handling	Environment	Easy	Easy	Easy	Easy	Medium	Hard	Very hard	–
Chemically defined diet	Environment	Yes	Yes	Yes/No	Yes	No	Yes/No	No	No

b **Experimental *C. elegans* holobiont model**



c **Metabolic *C. elegans* holobiont model**



Metabolic human holobiont model