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Review

Bacteriophages of the Human Gut: The "Known Unknown" of the Microbiome

Andrey N. Shkoporov[1,*] and Colin Hill[1]
[1]APC Microbiome Ireland & School of Microbiology, University College Cork, Co. Cork, Ireland
*Correspondence: andrey.shkoporov@ucc.ie
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The human gut microbiome is a dense and taxonomically diverse consortium of microorganisms. While the bacterial components of the microbiome have received considerable attention, comparatively little is known about the composition and physiological significance of human gut-associated bacteriophage populations (phageome). By extrapolating our knowledge of phage-host interactions from other environments, one could expect that >10^{12} viruses reside in the human gut, and we can predict that they play important roles in regulating the complex microbial networks operating in this habitat. Before delving into their function, we need to first overcome the challenges associated with studying and characterizing the phageome. In this Review, we summarize the available methods and main findings regarding taxonomic composition, community structure, and population dynamics in the human gut phageome. We also discuss the main challenges in the field and identify promising avenues for future research.

Introduction

The human body has been referred to as a "superorganism" (Aziz et al., 2013) in which microbial cells are present in numbers (~10^{14}) comparable to human cells (Sender et al., 2016). An overwhelming majority (>99%) of these microbes are located in the distal segments of the gastrointestinal tract (GIT). They occupy different ecological niches in the gut lumen and on mucosal surfaces, forming complex biochemical interaction networks between themselves and with the host organism. The dynamic equilibrium of the gut microbiome is essential for normal host physiology. For instance, gut microbes participate in host metabolic processes (Everard and Cani, 2013), stimulate normal development of immunity and brain functions in early ontogenesis (Dinan and Cryan, 2017), provide a barrier against incoming pathogens, and balance local immune responses throughout life (Belkaid and Hand, 2014). This has led to an appreciation of the human gut microbiome as a "forgotten organ," an essential, albeit genetically and antigenically foreign, component of the human body (O'Hara and Shanahan, 2006). The gut microbiome contains all three domains of cellular life, *Bacteria*, *Archaea*, and *Eukarya*, as well as viruses, albeit at very different relative concentrations (Figure 1). *Bacteria* and *Archaea* account for more than 99% of the unique characterized gene repertoire and biomass (Qin et al., 2010; Yatsunenko et al., 2012; Sender et al., 2016; Wampach et al., 2017) and have received most of the attention in human microbiome studies. At the same time, recent works have also highlighted the role of fungi and protozoa, microbial eukaryotes that constitute a smaller but potentially important part of the gut microbiome (Hoffmann et al., 2013; Huseyin et al., 2017; Laforest-Lapointe and Arrieta, 2018).

It is often postulated that viruses of bacteria are the most numerous biological entities on the planet and in many environments outnumber the counts of their prokaryotic hosts by a factor of 10 (Wommack and Colwell, 2000). The original hypothesis of linear virus-to-microbe ratio (VMR), based on early data from marine and freshwater microbial communities (Weinbauer, 2004; Thingstad et al., 2008), has been revised recently, with power law and unimodal models seeming to more accurately reflect extensive variation in VMR (2.6–160 in the oceans) (Knowles et al., 2016; Wigington et al., 2016). It has long been known that abundant and diverse communities of non-pathogenic viruses, mainly tailed bacteriophages, colonize the mammalian gut (Dhillon et al., 1976). Up until the last decade, however, the phageome remained the "known unknown" of the gut microbiome. This was mainly due to a very limited toolkit, which included direct observation and counting of virus-like particles (VLPs) using transmission electron (TEM) and epi-fluorescence microscopy (EFM) techniques, as well as isolation of individual bacteriophages infecting specific host strains in culture. Microscopic methods helped to reveal a large diversity of viral morphotypes (up to several tens per individual) with total counts of bacterial viruses in human feces, caecal contents, and colonic mucosa reaching ~10^9–10^{10} VLPs g^{-1} (Hoyles et al., 2014; Lepage et al., 2008). These were largely members of the *Caudovirales* order, represented by the *Siphoviridae*, *Podoviridae*, and *Myoviridae* families. Culture-based methods were mainly used to isolate bacteriophages against a limited set of model and clinically important microorganisms such as *Escherichia*/*Shigella* (Dhillon et al., 1976; Martinez-Castillo et al., 2013), *Enterococcus faecalis* (Bonilla et al., 2010), *Clostridioides difficile* (Hargreaves and Clokie, 2014), and a few other bacteria. Because >95% of bacteria residing in the distal gut, including non-pathogenic strict anaerobes belonging to families *Bacteroidaceae*, *Prevotellaceae*, *Ruminococcaceae*, *Lachnospiraceae*, etc., are difficult to culture, the available collections of phage strains of human fecal origin clearly still do not reflect the true diversity of human gut bacteriophages.

The advent of high-throughput metagenomic sequencing technology has allowed us to appreciate the complexity and richness of human gut bacteriophage populations (Breitbart et al., 2003, 2008). The first metagenomic studies of fecal viromes revealed that most bacterial viruses in the gut (81%–93%) are novel and can be neither assigned a taxonomic

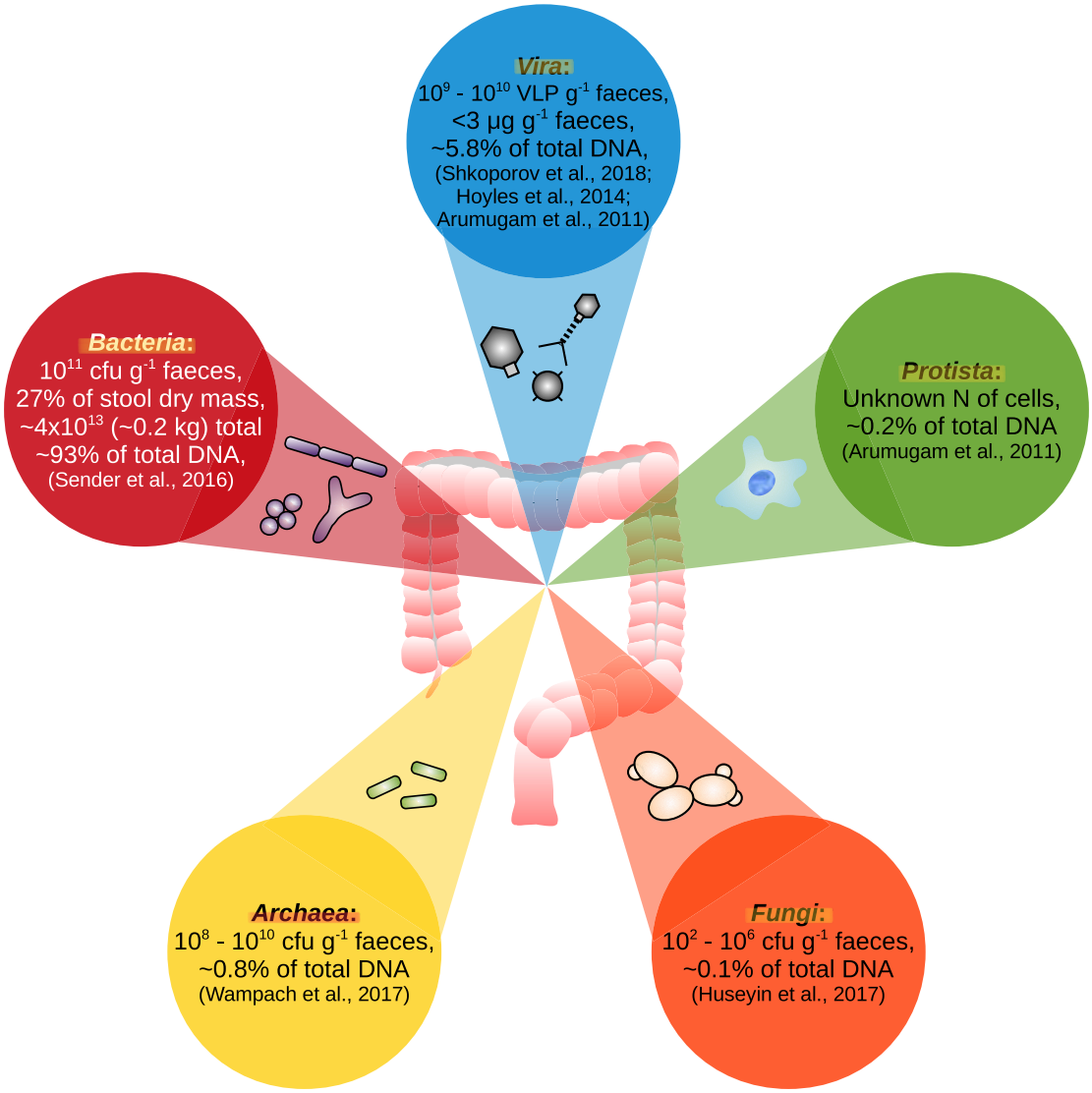

Figure 1. Main Taxonomic Groups of the Human Gut Microbiome and the Domain/Kingdom Level

position nor linked to a bacterial host (Manrique et al., 2016; Reyes et al., 2010). This is further complicated by the fact that human gut phageomes are highly individual specific, with only a small overlap between subjects (Manrique et al., 2016). The term "viral dark matter" has been coined to describe the existing gap in knowledge about the taxonomic composition and population structure of the gut phageome (Aggarwala et al., 2017). Description of the viral landscape in the gut would be incomplete without mentioning minority populations of circular, replication initiator protein (Rep) encoding, single-stranded DNA (CRESS-DNA) eukaryotic viruses (Lim et al., 2015; Reyes et al., 2015), and even pathogenic plant RNA viruses, which are likely of dietary origin but retain infectivity during transit through the gut (Zhang et al., 2006).

Widespread bacteriophage predation and lysogenic conversion in bacterial populations plays a major role in regulating bacterial biomass, maintaining biodiversity, horizontal gene transfer and driving biogeochemical cycles in the Earth biosphere (Thingstad et al., 2008). With phage-bacterial ratios of ~1:1 in the human gut (Carding et al., 2017), we can expect that bacteriophage predation, lysogeny, and gene transfer will play major roles in controlling the density, diversity, and network interactions inside gut-associated symbiotic bacterial communities as well. Importantly, specific and lasting changes of phageome composition were detected in a number of diverse gut-related and systemic conditions such as inflammatory bowel disease (IBD), malnutrition, and AIDS (Norman et al., 2015; Monaco et al., 2016; Reyes et al., 2015). Additionally, evidence of the efficacy of sterile fecal filtrate transfer in the treatment of *C. difficile* infection (CDI) points toward the potential ability of gut phages to restrict pathobiont growth and promote normal richness of the gut microbiota (Ott et al., 2017). Interestingly, however, the majority of gut bacteriophages seem to engage in lysogenic interactions with their hosts, thereby persisting for prolonged