

# UC Davis

## UC Davis Previously Published Works

### Title

Salmonella versus the Microbiome

### Permalink

<https://escholarship.org/uc/item/7rm4q4z6>

### Journal

Microbiology and Molecular Biology Reviews, 85(1)

### ISSN

1092-2172

### Authors

Rogers, Andrew WL  
Tsolis, Renée M  
Bäumler, Andreas J

### Publication Date

2021-02-17

### DOI

10.1128/mnbr.00027-19

Peer reviewed



# Salmonella versus the Microbiome

Andrew W. L. Rogers,<sup>a</sup>  Renée M. Tsolis,<sup>a</sup>  Andreas J. Bäumlér<sup>a</sup>

<sup>a</sup>Department of Medical Microbiology and Immunology, School of Medicine, University of California at Davis, Davis, California, USA

<b>SUMMARY</b> .....	1
<b>INTRODUCTION</b> .....	2
<b>THE GUT MICROBIOME</b> .....	3
Competition and Habitat Filtering Govern Gut Microbiota Assembly .....	3
Principles of community assembly .....	3
Habitat filtering establishes dominant taxa in the colonic microbiota .....	4
Competition and Habitat Filtering Maintain Microbiota Resistance and Resilience .....	5
Microbiota resistance .....	5
Microbiota resilience .....	7
Homeostasis versus dysbiosis .....	7
<b>COLONIZATION RESISTANCE</b> .....	7
A Host-Microbe Chimera Confers Colonization Resistance .....	7
Functions of the gut microbiota .....	7
Sterilizing immunity versus microbiota-nourishing immunity .....	8
Niche Modification by Microbiota-Derived Short-Chain Fatty Acids .....	9
Historical overview .....	9
Mechanism of growth inhibition by short-chain fatty acids .....	10
Short-chain fatty acid producers .....	10
Beyond short-chain fatty acids .....	11
Host-Derived Habitat Filters Uphold Colonization Resistance .....	11
Microbial signatures of dysbiosis .....	11
Host phagocytes transform the gut environment during inflammation .....	11
Epithelial metabolism shapes the gut microbiota .....	13
Niche Preemption by Endogenous <i>Enterobacterales</i> .....	14
Competition with closely related species .....	14
Keystone species limit the availability of critical resources .....	14
<b>NICHE OPPORTUNITIES CREATED BY VIRULENCE FACTORS</b> .....	15
Ecosystem Engineering by Virulence Factors Licenses Pathogen Engraftment .....	15
Pathogens overcome colonization resistance—opportunists do not .....	15
Virulence factors carve out a new nutrient niche for the pathogen .....	16
New niche opportunities create competition .....	17
Reconstructing <i>Salmonella</i> 's Nutrient Niche from the Ruins .....	18
Genome-guided assembly of a metabolic network for gut colonization .....	18
Predicting the metabolic landscape in <i>Salmonella</i> 's nutrient niche .....	21
<b>CONCLUDING REMARKS</b> .....	21
What <i>Salmonella</i> Serovars Teach Us about Dysbiosis .....	21
Where Do We Go from Here? .....	22
New strategies to rebalance the microbiota .....	22
Expanding the microbiome toolbox .....	23
<b>ACKNOWLEDGMENTS</b> .....	12
<b>REFERENCES</b> .....	23

**SUMMARY** A balanced gut microbiota contributes to health, but the mechanisms maintaining homeostasis remain elusive. Microbiota assembly during infancy is governed by competition between species and by environmental factors, termed habitat filters, that determine the range of successful traits within the microbial community. These habitat filters include the diet, host-derived resources, and microbiota-derived metabolites, such as short-chain fatty acids. Once the microbiota has matured, competition and habitat filtering prevent engraftment of new microbes, thereby providing protection against opportunistic infections. Competition with endogenous *Enterobacterales*, habitat filtering by short-chain fatty acids, and a host-derived habitat filter, epithelial hypoxia, also contribute to colonization resistance against *Salmonella* serovars. However, at a high challenge dose, these frank pathogens can overcome colonization resistance by

**Citation** Rogers AWL, Tsolis RM, Bäumlér AJ. 2021. *Salmonella* versus the microbiome. *Microbiol Mol Biol Rev* 85:e00027-19. <https://doi.org/10.1128/MMBR.00027-19>.

**Copyright** © 2020 American Society for Microbiology. All Rights Reserved.

Address correspondence to Andreas J. Bäumlér, [ajbaumler@ucdavis.edu](mailto:ajbaumler@ucdavis.edu).

**Published** 23 December 2020

using their virulence factors to trigger intestinal inflammation. In turn, inflammation increases the luminal availability of host-derived resources, such as oxygen, nitrate, tetra-thionate, and lactate, thereby creating a state of abnormal habitat filtering that enables the pathogen to overcome growth inhibition by short-chain fatty acids. Thus, studying the process of ecosystem invasion by *Salmonella* serovars clarifies that colonization resistance can become weakened by disrupting host-mediated habitat filtering. This insight is relevant for understanding how inflammation triggers dysbiosis linked to noncommunicable diseases, conditions in which endogenous *Enterobacterales* expand in the fecal microbiota using some of the same growth-limiting resources required by *Salmonella* serovars for ecosystem invasion. In essence, ecosystem invasion by *Salmonella* serovars suggests that homeostasis and dysbiosis simply represent states where competition and habitat filtering are normal or abnormal, respectively.

**KEYWORDS** colonization resistance, microbiome, microbiota, *Salmonella*

## INTRODUCTION

The idea that communicable diseases are due to infection with pathogens emerged with the inception of Louis Pasteur's germ theory in 1865 (1) and Robert Koch's invention of approaches to establish causality in 1882 (2). These game-changing discoveries became a guiding principle of a new discipline, bacteriology, which is the precursor of modern-day microbiology and immunology. Subsequent discoveries of diphtheria toxin in 1888 (3) and Shiga toxin in 1903 (4, 5) gave rise to the concept that pathogens cause disease because they elaborate virulence factors that manipulate host physiology. The century following these seminal discoveries has seen countless studies on how virulence factors enable pathogens to overcome host defenses in individuals with an intact immune system to cause disease. However, this historic focus on host pathogen interaction has left a third player in obscurity, our host-associated microbial communities, the microbiota.

Although research from the 1950s shows that a disruption of the microbiota enhances susceptibility to infection (6), the idea that virulence factors could play a role in overcoming growth inhibition by resident microbial communities was not explored until microbiota analysis became possible by advances in sequencing technologies during the first decade of the 21st century (7–9). Subsequent work shows that virulence factors can target the host to manipulate the environment inhabited by the microbiota (10–13). Through this chain of events, virulence factors can alter the microbiome, which is defined ecologically as the microbiota and its host environment (14, 15) (Table 1 and Fig. 1). The fact that mucosal pathogens can use virulence factors to manipulate the microbiome renders them useful tools for microbiome research (16). As a result, studies on how virulence factors manipulate the host/microbiota interface are beginning to assemble into a framework for a “new bacteriology” which studies pathogen physiology and gene regulation in the natural context of the microbiome (17).

Here, we will review this new chapter in bacteriology using the paradigm that spearheaded many advances: studies on the pathogenesis of *Salmonella* serovars. We will start by briefly outlining the conceptual framework of microbiome research, followed by discussing how the microbiome protects against infection and how *Salmonella* serovars use their virulence factors to overcome this line of defense. *Salmonella enterica* subspecies *enterica* serovar Typhimurium (*S. Typhimurium*) is commonly studied as a representative of the species, because it is an important human pathogen (18, 19). *S. Typhimurium* was first described as the causative agent of a typhoid-like disease in mice (20), a mammalian species that is commonly used to model the disease process (21). The luminal *S. Typhimurium* population reaches high numbers in the murine large intestine (22), which also harbors the largest microbial community in the human body. Most of our discussion will therefore revolve around the interaction of *S. Typhimurium* with the microbiota of the large intestine.

T1/F1

**TABLE 1** Microbiome vocabulary

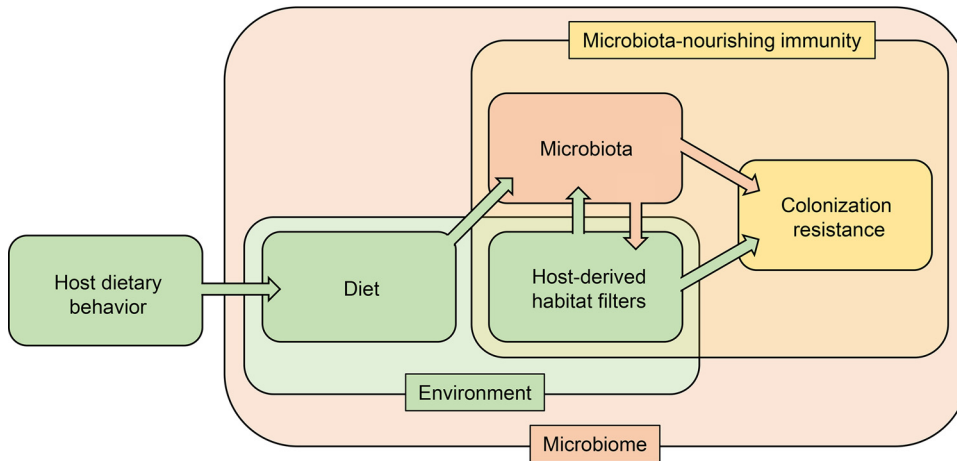
Term	Definition
Colonization resistance	Mechanisms executed by microbiota-nourishing immunity that prevent engraftment of a specific microorganism through competition and habitat filtering
Communicable disease	Disease caused by an infectious agent that is transmitted from one animal or person to another, through direct contact, or indirectly through fomites or vectors
Dominant taxa	The most abundant taxa in a community, exerting a strong influence on other taxa
Dysbiosis	A state of abnormal competition or habitat filtering
Ecosystem engineering	The process by which a keystone species modifies a habitat, thereby strongly effecting other organisms
Facultative anaerobic bacteria	Bacteria that can grow in the presence of (and often can respire) oxygen at atmospheric levels but can grow fermentatively when oxygen is absent
Foundation species	A species that provides the foundation for a habitat by physically modifying the environment, thereby structuring communities of other organisms
Habitat filters	Factors that select for microbial traits licensing growth and survival in a host habitat patch
Habitat patch	The dynamic environment on a host surface where the microbiota assembles
Historical contingency	Dependence of the taxon composition on the order and timing of species arrival during microbiota assembly
Homeostasis	The outcome of normal competition and normal habitat filtering, which in turn generates microbiota resistance and microbiota resilience
Keystone species	A species that has a disproportionately large effect on its habitat relative to its abundance within the microbial community
Microbiome	The microbiota and its host environment
Microbiota	Host-associated microbial communities
Microbiota-nourishing immunity	A subdivision of the immune system, composed of the microbiota and host-derived habitat filters, which confers colonization resistance on body surfaces
Microbiota resilience	The ability of the microbiota to return to a healthy equilibrium state after perturbation
Microbiota resistance	Temporal stability in the taxon composition of mature host-associated microbial communities
Niche modification	A mechanism that uses microbiota-mediated habitat filtering to prevent engraftment of microorganisms that harbor inadequate trait combinations
Niche preemption	A mechanism that uses direct competition for critical resources with members of the microbiota to prevent engraftment of similar microorganisms
Noncommunicable disease	Medical condition that is not caused by an infectious agent but is due to an underlying defect in host physiology that is not transmissible
Nutrient niche	An ecological position defined by critical resources that support growth of a suitable occupant
Obligate anaerobic bacteria	Bacteria that cannot respire oxygen and cannot grow under atmospheric oxygen concentrations
Opportunistic infection	Infection with opportunistic pathogen
Opportunistic pathogens	Microbes associated with disease in immunocompromised members of a host species
Pathogens (or frank pathogens)	Microbes associated with communicable diseases in immunocompetent members of a host species
Priority effects	The ability of resident microbes to prevent engraftment of new microorganisms through niche preemption and/or niche modification
Sterilizing immunity	The part of our immune system that preserves tissue sterility by detecting and distinguishing microbial intruders from self and subsequently triggering innate and adaptive immune responses aimed at removing the intruder from tissue
Virulence factors	Molecules produced by pathogens to overcome host defenses and cause disease

## THE GUT MICROBIOME

### Competition and Habitat Filtering Govern Gut Microbiota Assembly

**Principles of community assembly.** The infant is thought to be sterile *in utero* (23), suggesting that birth marks the beginning of microbiota assembly. According to ecological theory of plant community assembly, this process is governed by two drivers: competition and habitat filtering. In plant communities, competition involves interactions among species whereas habitat filtering encompasses interactions between species and their abiotic environment (24). These assembly rules also apply to the human gut microbiota (25, 26), except here the host provides a biotic environment that responds dynamically to microbiota-derived signals, which adds additional layers of complexity.

The host could be viewed as an ecological foundation species (27), who filters the habitat of the gut microbiota using biotic factors, which include physical barriers (e.g., peristalsis), the emission of chemicals (e.g., gastric acid and bile acids), the excretion of antimicrobial proteins (e.g., defensins), the secretion of immunoglobulin A (IgA), and epithelial release of resources that shape microbial growth (e.g., mucin) (28, 29). In addition to host-derived habitat filters, the range of successful traits within the



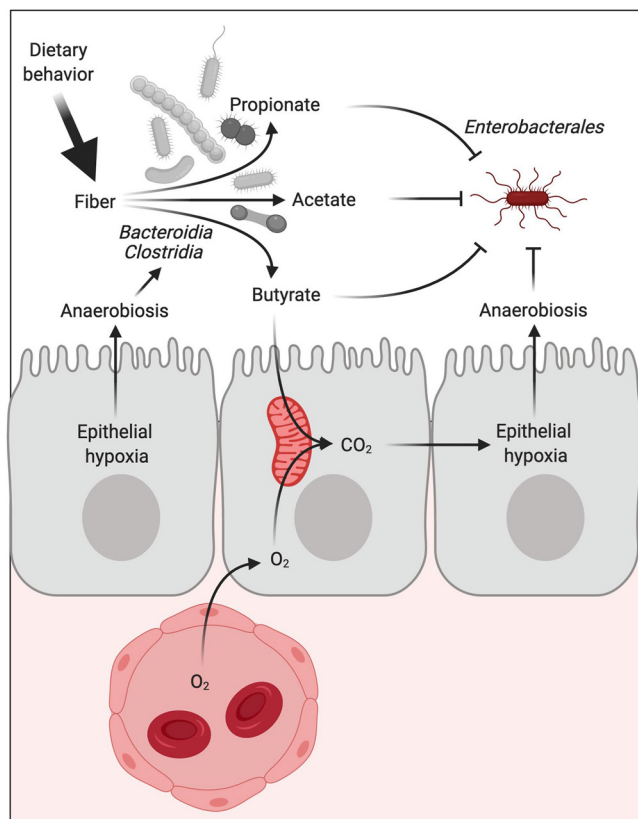
**FIG 1** Composition of the term microbiome. The microbiome is defined as the microbiota and its environment. The latter is determined by host-derived habitat filters and the diet, which is controlled by host behavior. Host-derived habitat filters shape the size, species composition and biogeography of the microbiota and in turn the microbiota contributes to host nutrition and immune education. Microbiota-nourishing immunity is composed of the microbiota and host-derived habitat filters, which form a host-microbe chimera that functions in conferring colonization resistance.

microbiota in the gastrointestinal tract is influenced by microbiota-derived habitat filters (e.g., short-chain fatty acids) and by an important abiotic habitat filter: the diet (30). Since the choice of diet is governed by host behavior, diet could also be viewed as an aspect of host-mediated habitat filtering (Fig. 1).

Competition and habitat filtering select for different functional traits of coexisting species. Competition is common among pairs of similar species and can lead to competitive exclusion, thereby limiting the number of similar coexisting species (31). In contrast, habitat filtering limits the range of successful strategies among coexisting species (32), which can drive species with particular traits or phenotypes to dominate the microbial community.

**Habitat filtering establishes dominant taxa in the colonic microbiota.** One important host-derived habitat filter that shapes the abundance of species inhabiting the colon is epithelial hypoxia. The healthy colonic epithelium permanently resides in a state of physiological hypoxia (<1% oxygen) (33), which limits the amount of oxygen diffusing into the lumen of the colon, thereby maintaining anaerobiosis (34). As a result, obligate anaerobic bacteria dominate the microbial community in the colon (35), a phenotypic convergence in a key ecological trait. Elevating epithelial oxygenation disrupts this biotic habitat filter, thereby increasing oxygen availability in the intestinal lumen, which results in an expansion of facultative anaerobic bacteria in the colonic microbiota (36), a microbial signature of dysbiosis (37).

A second important habitat filter for the colonic microbiota is the diet. Milk oligosaccharides in breast milk represent an important maternal habitat filter, as these dietary carbohydrates do not nurture the infant, are poorly absorbed in the small intestine, and reach the colon (38). Milk oligosaccharides drive a predominance of *Bifidobacteriaceae* (phylum *Actinobacteria*) because these obligate anaerobes are among a select few bacteria that contain gene clusters for the consumption of these carbohydrates (39, 40). Weaning removes human milk oligosaccharides from the diet while introducing dietary fiber, an important habitat filter involved in shaping the colonic microbiota. Dietary fiber is composed of complex carbohydrates that are not degraded and absorbed by host enzymes in the upper gastrointestinal tract, thus making them available as carbon sources for the colonic microbiota (41, 42). Phenotypic traits conferring the ability to utilize dietary fiber are most abundant in members of the classes *Clostridia* (phylum *Firmicutes*) and *Bacteroidia* (phylum *Bacteroidetes*) (43). As a result, weaning is associated with a succession characterized by a



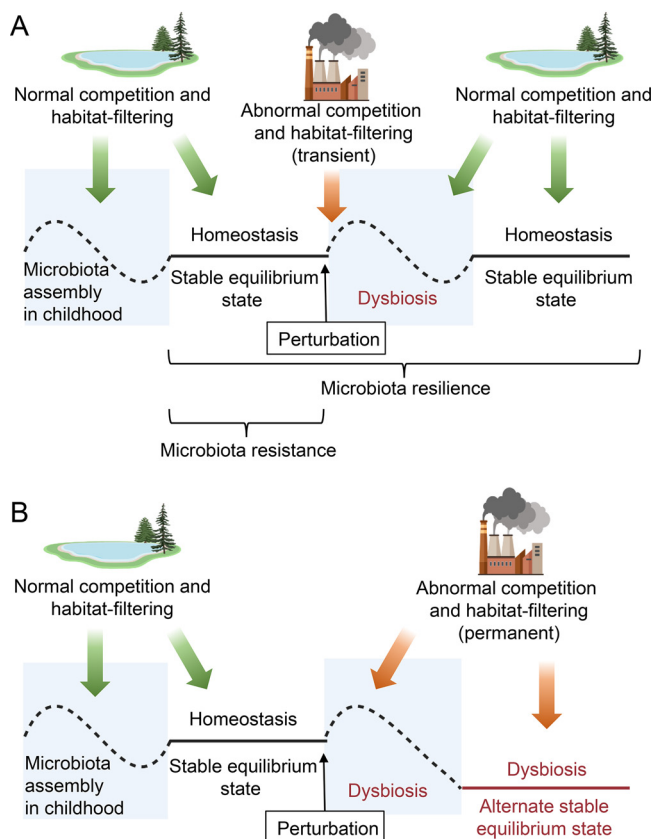
**FIG 2** Habitat filtering in the adult colon. Epithelial hypoxia and dietary fiber filter the habitat in the large intestine to license growth of obligate anaerobic fiber eaters, which drives a dominance of the classes *Clostridia* and *Bacteroidia* in the fecal microbiota. Facultative anaerobic bacteria, such as members of the *Enterobacteriales*, remain minority species because epithelial hypoxia limits critical resources they require for overcoming growth inhibition by short-chain fatty acids (acetate, butyrate, and propionate). (Created with BioRender.com.)

disappearance of *Bifidobacteriaceae* and an expansion of *Clostridia* and *Bacteroidia* in the gut microbiota (44).

These observations illustrate that the dominance of certain bacterial taxa in the colonic microbiota is the result of habitat filtering by the host, which involves the host's dietary behavior and host control over the flow of resources from the epithelial lining into the microbial habitat. In other words, dietary fiber and epithelial hypoxia "filter" the colonic environment in healthy adults so that obligate anaerobic bacteria with a diverse array of glycolytic enzymes predominate, which explains why *Clostridia* and *Bacteroidia* are the most abundant taxa in this habitat patch (35) (Fig. 2).

### Competition and Habitat Filtering Maintain Microbiota Resistance and Resilience

**Microbiota resistance.** As the microbiota matures, ecological niches carved out through competition and habitat filtering become successively occupied by microorganisms that are acquired stochastically over time from maternal or environmental sources (45). Fecal microbiota transplantation in adult mice increases species diversity compared to the microbiota of both the donor and the recipient, which suggests that the microbiota assembly process does not reach full saturation (46), a property common to most ecosystems (47, 48). Nonetheless, established members of the microbial community can prevent engraftment of new arrivals either through competition, a process known as niche preemption, or through habitat filtering, an activity referred to as niche modification (45, 49). Niche preemption can involve competition between closely related species for critical resources, such as oxygen (50, 51). An example of niche modification is the production of short-chain fatty acids by



**FIG 3** Normal competition and habitat filtering promote homeostasis, microbiota resistance and microbiota resilience. (A) After birth, the microbiota exhibits fluctuations as it assembles to fill nutrient niches created by competition and habitat filtering. Once microbiota assembly is complete, a state of normal competition and habitat filtering maintains homeostasis, characterized by a stable equilibrium state in which the microbiota composition remains invariable over time, a phenomenon termed microbiota resistance. A brief perturbation, such as a disruption of the microbiota with antibiotics, leads to a transient state of abnormal competition and habitat filtering, which causes dysbiotic fluctuation in the microbiota composition. However, once normal competition and habitat filtering resume, the microbiota reassembles to reach an equilibrium state that is functionally similar to that of the community prior to the perturbation. The ability of the microbiota to return to homeostasis after a perturbation is termed microbiota resilience. (B) A lasting perturbation, which can be caused for example by chronic intestinal inflammation, triggers a permanent state of abnormal competition and habitat filtering. As new nutrient niches created by abnormal competition and habitat filtering are filled, the microbiota composition shifts permanently to reach an alternate equilibrium state. Through this process, abnormal competition and habitat filtering maintain a perpetual state of dysbiosis. (Created with BioRender.com.)

*Clostridia* and *Bacteroidia* species (52, 53), which limits the range of successful metabolic strategies among bacterial species inhabiting the large intestine. Niche pre-emption and niche modification generate priority effects that enable founding members of a mature microbial community to prevent engraftment of additional microbes, thereby generating a stable equilibrium state with invariable species composition (54, 55). The resulting temporal stability of the taxon composition observed for mature gut-associated microbial communities is termed microbiota resistance (45, 56, 57) (Fig. 3A).

Since historical events that govern the initial exposure to microbes differ between individuals, the outcome of community assembly is different for each person, a phenomenon known as historical contingency (49). Combining historical contingency with microbiota resistance is predicted to generate considerable taxonomic diversity between gut-associated microbial communities from different individuals. Consistent with this idea, the taxon composition exhibits little overlap on the species level when the fecal microbiota composition is compared between healthy volunteers (58).



**Microbiota resilience.** The principles of microbial community assembly predict that competition and habitat filtering will select for comparable microbial traits in healthy individuals that consume a similar diet, which will result in assembly of microbial communities that are functionally similar even though they differ in their species composition. Consistent with this idea, antibiotics disrupt the fecal microbiota by permanently removing some microorganisms, but after completing therapy, habitat filtering ensures that vacated niches are occupied again by microbes harboring traits similar to those of their predecessors, thus returning the microbiota to a healthy equilibrium state despite the fact that recovery from antibiotic treatment changes the species composition (54). For example, oral administration of streptomycin diminishes microbial functions, such as short-chain fatty acid production, but concentrations of these metabolites return to normal levels after cessation of treatment (59), suggesting that reassembly of the microbiota returns metabolic traits to their ancestral state. Through this mechanism, competition and habitat filtering ensures that the microbiota returns to a healthy state after perturbation, a property called microbiota resilience (56, 60) (Fig. 3A).

**Homeostasis versus dysbiosis.** The taxonomic diversity in the microbiota composition between individuals (58) makes it all but impossible to determine what constitutes a balanced microbial community based on cataloguing microbial species names (61). Dysbiosis is commonly described as an imbalance in microbial communities characterized by a decrease in microbial diversity, the presence of potentially harmful microbes or the absence of beneficial ones (62), but this definition becomes untenable when homeostasis cannot be explained by the presence or absence of specific microbial species (63). Problems with a taxonomic definition for homeostasis and dysbiosis provide a compelling rationale for developing functional definitions for these terms (27). The processes that govern microbial community assembly suggest that homeostasis represents the outcome of normal competition and habitat filtering, which in turn generates microbiota resistance and microbiota resilience. Normal habitat filtering could be defined as an activity characteristic of or appropriate to a healthy or normally functioning host. Conversely, dysregulation of processes involved in microbial community assembly will trigger dysbiosis, which can be defined as a state resulting from abnormal competition or habitat filtering (Fig. 3B).

## COLONIZATION RESISTANCE

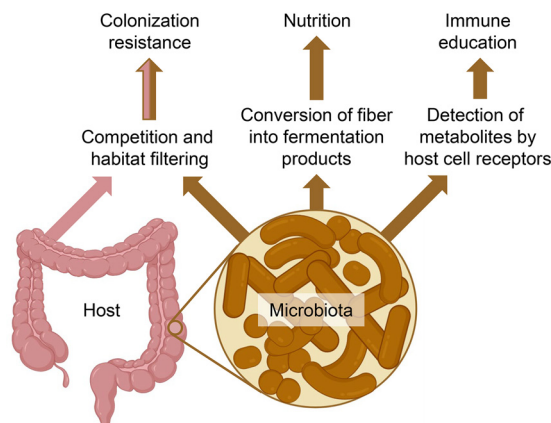
### A Host-Microbe Chimera Confers Colonization Resistance

**Functions of the gut microbiota.** Defining homeostasis functionally focuses attention on the role the gut microbiota plays in health. One function of a balanced colonic microbiota is to aid in the digestion of nutrients that cannot be broken down by host enzymes in the small intestine, such as fiber (64). Habitat filters that maintain anaerobiosis ensure that catabolism of fiber has to proceed through pathways that generate fermentation products, such as short-chain fatty acids (65). In turn, microbiota-derived fermentation products are absorbed by the host for nutrition, which provides us with an estimated 6 to 10% of our energy budget (66, 67). The microbiota has thus been likened to an organ containing our “second genome,” which encodes digestive enzymes to harvest otherwise inaccessible nutrients (68, 69) (Fig. 4).

A second function of a balanced gut microbiota is to educate and prime our host defenses (70–76). Altered production of microbiota-derived metabolites during dysbiosis has been linked to a broad spectrum of noncommunicable diseases associated with chronic immune activation, such as colorectal cancer (77), atherosclerosis (78) and allergic airways disease (79). Comparison of germfree and conventional mice reveals that the microbiota profoundly influences functionality and development of both the mucosal and systemic immune systems (80, 81). It has thus been proposed that the microbiota should be viewed as an organ aiding in immune education (82) (Fig. 4).

The organ analogy has obvious limitations, as organs are passed down across generations, whereas heritability estimates for the human microbiota are low (83, 84).





**FIG 4** Functions of the gut microbiota. Nutrients (e.g., fiber) that evade absorption and degradation by host enzymes in the small intestine enter the colon, where they are converted into fermentation products by the gut microbiota. This metabolic activity of the gut microbiota has been likened to the function of an organ that contributes to host nutrition and immune education. Host-derived habitat filters and the microbiota form a host microbe chimera that performs a third function, termed colonization resistance, which prevents harmful microbes from entering the body. (Created with BioRender.com.)

Furthermore, the fact that germfree mice are viable suggests that the digestive function of the microbiota and its role in immune education are not essential for life. However, one could argue that the latter assertion is flawed, because germfree mice require dietary supplementation with microbial products (e.g., vitamin K) (85) and are exquisitely sensitive to infection. In the absence of a microbiota, environmental exposure would inevitably result in death from opportunistic infections. These considerations underscore that in addition to aiding nutrition and immune education, the microbiota executes a third function that contributes to health, which is to limit the ability of harmful microbes to gain a foothold and expand on body surfaces, a property known as colonization resistance (Fig. 4). Colonization resistance is a canonical nonspecific immune function that is essential for life. This vantage point suggests that our resident microbes should be considered effector cells of our immune system, an idea that requires an expansion of theory to incorporate microbial ecology into the classical framework of immunology (28).

**Sterilizing immunity versus microbiota-nourishing immunity.** One subdivision of our immune system ensures sterility of host tissues (sterilizing immunity) by detecting invading microorganisms and distinguishing them from self. In turn, self/nonself discrimination induces innate and adaptive immune responses that are aimed at removing the microbial intruders from tissue to restore sterility (86). However, whereas the goal of sterilizing immunity is to remove microbes from host tissues, the goal of our interaction with microbes inhabiting body surfaces is not to detect and remove them but rather to maintain and balance microbial communities for health (87). It has thus been proposed that host-derived habitat filters that shape microbial communities form a functional unit with the microbiota, termed microbiota-nourishing immunity, which constitutes an immune system subdivision that is separate from sterilizing immunity (28, 88) (Fig. 1).

Several fundamental differences between microbiota-nourishing immunity and sterilizing immunity justify such a subdivision. First, at the very core of sterilizing immunity lies the ability to discriminate between self and nonself, which needs to be applied to members of the microbiota. For instance, microbiota entering tissue during traumatic injury necessitates its elimination to restore sterility. However, whereas self/nonself discrimination by sterilizing immunity is essential for hunting down individual microbes in host tissue, this process is not critical for balancing the microbiota on body surfaces using host-derived habitat filters. Although microbiota-derived

metabolites can be detected by host cell receptors to regulate host-derived habitat filters (89–91), this process neither distinguishes individual microbes from self, nor does it trigger responses aimed at sterilizing body surfaces (92, 93).

Second, microbiota-nourishing immunity is a host-microbe chimera, in which the microbial contribution to colonization resistance is mediated through ecological priority effects executed by microbial effector cells (94). In contrast, all components involved in sterilizing immunity are host derived, which makes the idea that microbial cells could be considered effector cells of our immune system appear strange to card-carrying immunologists (28).

Third, although there is overlap between antimicrobial mechanisms employed by effector cells of sterilizing immunity and by host-derived habitat filters of microbiota-nourishing immunity (e.g., defensins), only the latter employs mechanisms that literally nourish the microbiota (e.g., milk oligosaccharides) (28). Thus, habitat filters of microbiota-nourishing immunity balance the microbiota using a carrot-and-stick approach that is never utilized by sterilizing immunity.

The emerging picture suggests that microbiota-nourishing immunity constitutes our first line of defense against mucosal pathogens, but our functional understanding of this immune system subdivision lags behind that of sterilizing immunity. Although the concept of microbiota-nourishing immunity is new (28, 88), there is a large body of work on colonization resistance reaching all the way back to the 1950s (6). Taking a fresh look at this literature through the novel lens of microbiota-nourishing immunity provides an opportunity to infuse the conceptual framework of a data-driven discipline, microbiome research, with a wealth of information on bacterial physiology and pathogenesis. Here, we will perform this task for *S. Typhimurium*, one the best-studied bacterial model organisms that has long been a workhorse of research in bacterial genetics and metabolism (95). *S. Typhimurium* is ideally suited for studying the interplay between the pathogen, the host, and its microbiota due to the availability of excellent animal models (21, 96).

### Niche Modification by Microbiota-Derived Short-Chain Fatty Acids

**Historical overview.** A clinical appreciation for the protective functions of the gastrointestinal microbiota began in the 1940s and 1950s with the rapid introduction of antibiotics for the treatment of bacterial infections (97). Alongside the profound success of antibiotic therapies came the observation that patients often became susceptible to secondary bacterial infections after antibiotic treatment of a primary infection, implicating an unperturbed microbiota as a key player in the generation of colonization resistance (98–100). In an effort to study this phenomenon, Marjorie Bohnhoff at the University of Chicago pioneered the use of a mouse model for the study of microbiota-mediated colonization resistance (6). This model, which is still widely used today, involves oral pretreatment of mice with the antibiotic streptomycin. The treatment was found to significantly alter the abundance and composition of the large intestinal microbiota, measured by the contemporary standards of aerobic plate counts and Gram stain (101, 102). An acute susceptibility to intragastric *S. Enteritidis* infection coincides with this streptomycin-dependent alteration of the large intestinal microbiota, with the infectious dose being lowered to <10 CFU, whereas untreated mice resist colonization by *S. Enteritidis* challenges of as high as  $10^6$  CFU (6). This 10,000-fold increase in the challenge dose required for lethal *S. Enteritidis* infection in mice with an intact microbiota compared to mice with streptomycin-ablated microbiota illustrates that colonization resistance provides strong protection against low-dose pathogen challenge. Early work also contributed a prescient description of microbiota resilience, by demonstrating that a drastic reduction in the overall bacterial abundance and morphological diversity triggered by streptomycin treatment rebounded to pretreatment levels within 1 week (101, 102).

Initial studies on streptomycin-pretreated mice suggest that an intact microbiota has bacteriostatic or weakly bactericidal activity against *S. Typhimurium*, which is attributable to the metabolic functions of the microbiota (52). The most abundant by-

products of the fermentative metabolism of the colonic microbiota are the short-chain fatty acids acetate, propionate, and butyrate. Fecal concentrations of acetate are commonly measured in the 50 mM range, while propionate and butyrate levels vary widely from 5 to 30 mM. The observation that short-chain fatty acids are required to inhibit the growth of *S. Enteritidis* *in vivo*, in fecal homogenates *ex vivo*, and in rich media *in vitro* reveals their crucial role in mediating colonization resistance (53). However, the inhibitory mechanism of action of short-chain fatty acids requires an acidic environmental pH since only their protonated forms exhibit significant inhibitory effects on the growth of members of the *Enterobacterales* (*ord. nov.* [103]) the order *Salmonella* serovars belong to, by freely diffusing across cellular membranes (104). Initial characterization of the large intestinal environment of mice revealed that short-chain fatty acids are present at high concentrations alongside a mildly acidic pH (53, 101). Disturbance of the microbiota by streptomycin treatment lowers short-chain fatty acid concentrations and increases the luminal pH of the large intestine, thereby generating conditions that are favorable to growth of *Salmonella* serovars *in vivo* and *in vitro* (53, 101).

However, the inhibitory activity of short-chain fatty acids alone is not sufficient to explain how a low abundance of *Enterobacterales* is maintained in the microbiota because this would require short-chain fatty acids to be preset constantly at precisely the right concentration to check population growth, whereas any further increases in the concentration would drive this taxon to extinction (105). Rolf Freter thus proposed that the abundance of *Enterobacterales* in the fecal microbiota is determined by the availability of growth-limiting resources (106). During homeostasis, a low abundance of these growth-limiting resources maintains *Enterobacterales* as minority species in the microbiota (106). A first inkling of the possible nature of these growth-limiting resources comes from the early observation that depletion of short-chain fatty acids increases the redox potential in the cecum to conditions that approximate an aerobic broth culture (101), which predates the finding that streptomycin increases oxygen availability in the colon by more than 50 years (89). A more detailed discussion of growth-limiting resources that govern the abundance of *Enterobacterales* in the fecal microbiota is provided below in the section on host-derived habitat filters.

**Mechanism of growth inhibition by short-chain fatty acids.** More recent work on colonization resistance against *Salmonella* serovars and other *Enterobacterales* confirms the importance of short-chain fatty acids and provides mechanistic insights into their mode of growth inhibition (107–109). As weak acids, the degree of dissociation for acetate, propionate, and butyrate decreases as the environmental pH approaches their respective  $pK_a$  values (the negative base 10 logarithm of the acid dissociation constant) of 4.76, 4.87, and 4.82. In order to maintain a proton motive force and cellular homeostasis, *Enterobacterales* maintain their intracellular pH in the range of 7.2 to 7.8 (110–113). This cytosolic pH range is essential for driving ATP production by oxidative phosphorylation, which relies on protons translocating through ATP synthase and down their concentration gradient. When protonated short-chain fatty acids (HAc) diffuse into a bacterial cell, intracellular proton release ( $H^+ + Ac^-$ ) disrupts pH homeostasis in the cytosol (101, 110, 114, 115). If enough protonated short-chain fatty acids are present in the environment, then this process will proceed until the intracellular pH matches the environmental pH, thereby disturbing cellular pH homeostasis (108). Therefore, the inhibitory capacity of short-chain fatty acids is determined both by their concentration and the luminal pH, which is described by the Henderson-Hasselbalch equation ( $pH = pK_a + \log_{10}\{[Ac^-]/[HAc]\}$ ). Through this mechanism, short-chain fatty acids act as a habitat filter that maintains a low abundance of *Enterobacterales* during homeostasis.

**Short-chain fatty acid producers.** Acetate is produced by a broad range of bacterial species and cannot be attributed to a specific taxon within the gut microbiota. In contrast, *Bacteroidaceae* (class *Bacteroidia*) are the main producers of propionate (107), whereas the bulk of butyrate production in the colon is attributed to *Ruminococcaceae*

(class *Clostridia*) and *Lachnospiraceae* (class *Clostridia*) (116, 117). Variation in the abundance of *Bacteroidaceae* between different inbred mouse lines reveals that propionate production by members of this family contributes to colonization resistance against *S. Typhimurium* (107). Colonization resistance in mice harboring a gut microbiota with low *Bacteroidaceae* abundance can be strengthened by administering *Bacteroides thetaiotaomicron*, but not a *B. thetaiotaomicron* mutant deficient for propionate production (107). Similarly, a streptomycin-mediated depletion of *Ruminococcaceae* and *Lachnospiraceae* weakens colonization resistance against *S. Typhimurium*, which can be restored by administering butyrate or butyrate-producing *Clostridia* isolates (89, 118).

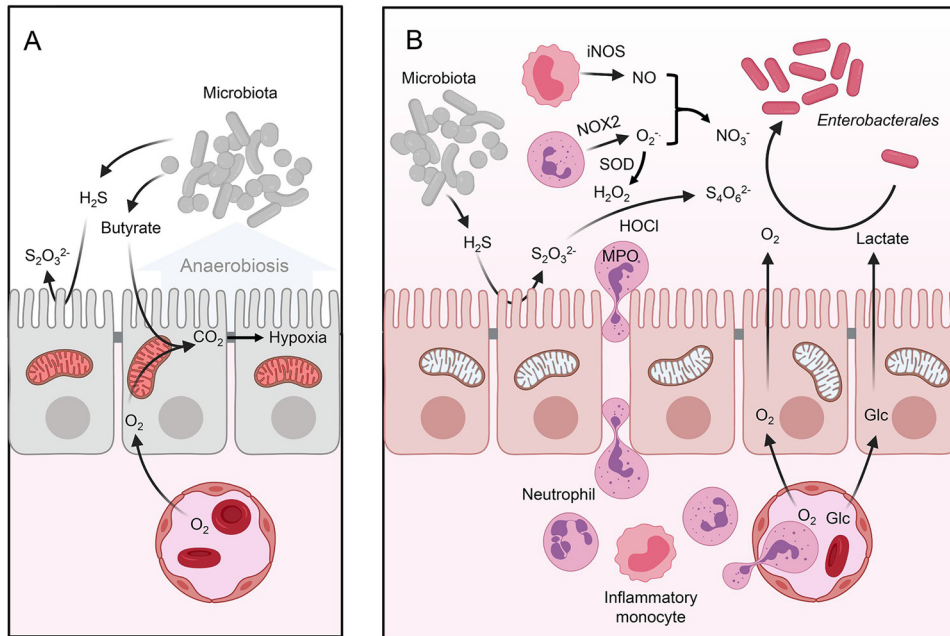
**Beyond short-chain fatty acids.** Recent work suggests that suppression of *S. Typhimurium* growth by microbiota-mediated habitat filtering is not limited to the production of short-chain fatty acids, but also includes a depletion of critical resources, such as amino acids. A microbiota-mediated depletion of amino acids filters the environment to exclude bacteria that lack amino acid biosynthesis pathways (119), a selective pressure that helps maintain prototrophy in *S. Typhimurium*. This selective pressure no longer acts on *Salmonella* serovars that are exclusively associated with extraintestinal disease, such as the human-adapted *S. Typhi* or *S. Paratyphi A* (120), which might explain why these pathogens are auxotrophic for tryptophan or cysteine and arginine, respectively (121, 122). Depletion of the gut microbiota with antibiotics increases the concentrations of amino acids in the colonic lumen (123). In turn, *S. Typhimurium* can take advantage of the increased availability of amino acids after antibiotic treatment by utilizing aspartate as an exogenous electron acceptor for fumarate respiration (124).

### Host-Derived Habitat Filters Uphold Colonization Resistance

**Microbial signatures of dysbiosis.** *Salmonella* serovars belong to the order *Enterobacterales* (ord. nov. [103], phylum *Proteobacteria*), a taxon comprising less than 0.1% of the human fecal microbiota in healthy volunteers (35). However, disruption of the microbiota during antibiotic therapy weakens colonization resistance, which gives rise to a dysbiotic expansion of endogenous *Enterobacterales* in the fecal microbiota (125). Hence, the main experimental approach for studying colonization resistance against *Enterobacterales* has been to disrupt the microbiota using antibiotics, causing the majority of studies to become fixated on microbial factors contributing to this nonspecific immune function. As a result, conventional wisdom, summarized in a number of recent review articles (126–130) stipulates that colonization resistance is mediated solely by the gut microbiota through a “battle of the bugs,” a process that does not involve the host.

However, the advent of microbiome research is beginning to shift this paradigm by revealing that in addition to the microbiota, the host makes important contributions to colonization resistance against *Enterobacterales* (Fig. 4) (34, 131). During homeostasis, anaerobiosis in the large intestine maintains a dominance of obligate anaerobic bacteria (Fig. 5A). However, profiling of the human fecal microbiota reveals that an expansion of facultative anaerobic *Enterobacterales* is one of the most consistent and robust ecological patterns associated with dysbiosis (37), which is commonly observed in the absence of antibiotic therapy. For example, this microbial signature of dysbiosis is associated with chronic alcohol consumption (132), radiotherapy (133), malnutrition (134), and inflammaging (chronic, sterile, low-grade inflammation associated with aging) (135) and is observed in individuals with inflammatory bowel disease (IBD) (136), colorectal cancer (77), necrotizing enterocolitis (137), HIV enteropathy (138), graft-versus-host disease (139), and infectious diarrhea (140). There is now mounting evidence that in many of these diseases, a dysbiotic expansion of *Enterobacterales* in the fecal microbiota is driven by an underlying dysregulation of host-mediated habitat filtering.

**Host phagocytes transform the gut environment during inflammation.** Studies on *Salmonella* pathogenesis spearheaded this research by showing that, paradoxically, severe acute intestinal inflammation drives a pathogen expansion in the gut microbiota (7, 8), in part because phagocytes migrating into the intestinal lumen release



**FIG 5** Intestinal inflammation creates a state of abnormal habitat filtering. (A) During homeostasis, microbiota-derived butyrate maintains high oxygen (O<sub>2</sub>) consumption in the colonic epithelium through mitochondrial oxidative phosphorylation (246–248). The resulting epithelial hypoxia limits diffusion of oxygen into the gut lumen to preserve anaerobiosis, which maintains a dominance of obligate anaerobic bacteria in the gut microbiota (89). Sulfate-reducing bacteria generate hydrogen sulfide (H<sub>2</sub>S) (249), which is detoxified by epithelial sulfide oxidases to thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>) (250, 251). (B) During intestinal inflammation, neutrophils and inflammatory monocytes migrate into the intestinal lumen. Inflammatory monocytes are the dominant source of inducible nitric oxide synthase (iNOS), which generates nitric oxide (NO) (252). Nitric oxide can react with superoxide (O<sub>2</sub><sup>-</sup>) produced by phagocyte NADPH oxidase (NOX2) to form peroxynitrite (253), which decomposes to nitrate (NO<sub>3</sub><sup>-</sup>) in the gut lumen (254). Superoxide is converted by superoxide dismutase (SOD) to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which is converted to hypochloric acid (HOCl) by neutrophil myeloperoxidase (MPO). These reactive oxygen species oxidize thiosulfate to tetrathionate (S<sub>4</sub>O<sub>6</sub><sup>2-</sup>) (10). Intestinal inflammation reduces mitochondrial bioenergetics in the colonic epithelium, thereby reducing epithelial oxygen consumption (154). The resulting loss of epithelial hypoxia increases diffusion of oxygen into the intestinal lumen to disrupt anaerobiosis. Catabolism of glucose (Glc) by host cells through aerobic glycolysis increases the luminal concentration of host-derived lactate (59). Through these mechanisms, intestinal inflammation elevates the availability of oxygen, lactate, nitrate and tetrathionate in the colonic lumen to create a state of abnormal habitat filtering that drives an expansion of facultative anaerobic *Enterobacteriales*, which is a microbial signature of dysbiosis in the fecal microbiota (36, 37). (Created with BioRender.com.)

antimicrobial compounds, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) (10, 141). Although direct exposure to these antimicrobial compounds can kill the pathogen (142, 143), phagocyte-derived ROS and RNS diffuse into the gut lumen, where they react to form nontoxic by-products, such as tetrathionate and nitrate, which serve as electron acceptors for anaerobic respiration, thereby promoting *S. Typhimurium* growth (10, 141, 144, 145) (Fig. 5B). Subsequent work shows that intestinal inflammation also weakens colonization resistance against other members of the *Enterobacteriales* through similar mechanisms. For instance, intestinal inflammation triggered by virulence factors of the enteric pathogen *Yersinia enterocolitica* causes the pathogen to expand in the gut microbiota through tetrathionate respiration (12). Similarly, nitrate respiration drives a dysbiotic expansion of commensal *E. coli* (order *Enterobacteriales*) in mouse models of *Toxoplasma gondii*-induced colitis (146), chemically induced colitis (147, 148), or genetically induced colitis (147, 148). Host-derived nitrate also weakens colonization resistance against *Klebsiella oxytoca* in a mouse model of cancer cachexia (149). In conclusion, migration of phagocytes into the intestinal lumen during intestinal inflammation lowers colonization resistance against *Enterobacteriales* by inducing a state of abnormal habitat filtering, which creates increased luminal concentrations of electron acceptors that drive an expansion of facultative anaerobic bacteria through anaerobic respiration (131).



**Epithelial metabolism shapes the gut microbiota.** Research on *Salmonella* pathogenesis was also at the forefront of discovering that the metabolism of the colonic epithelium functions as a control switch, mediating a shift between homeostatic and dysbiotic microbial communities (34). Virulence factors of *S. Typhimurium* trigger a shift in epithelial energy metabolism from mitochondrial oxidative phosphorylation to aerobic glycolysis, thereby reducing epithelial oxygen consumption in the colonic epithelium (118). The resulting loss of epithelial hypoxia increases the amount of oxygen diffusing into the intestinal lumen, thereby disrupting anaerobiosis and driving a pathogen expansion through aerobic respiration (50, 59, 118) (Fig. 5B). Loss of epithelial hypoxia in the colon is also induced by virulence factors of *Citrobacter rodentium*, a murine pathogen that expands in the colonic microbiota by exploiting the resulting increase in luminal oxygen bioavailability to fuel its growth (9, 11, 150, 151). Reduced mitochondrial bioenergetics in the colonic epithelium not only are linked to aerobic growth of enteric pathogens but also contribute to a weakening of colonization resistance against commensal *Enterobacterales* in mouse models of noncommunicable diseases, such as ulcerative colitis (152, 153) or colorectal cancer (154). Collectively, these data suggest that physiological hypoxia of the colonic surface limits growth of *Enterobacterales* during homeostasis (34, 36). However, conditions that reduce mitochondrial bioenergetics in the colonic epithelium increase the luminal availability of host-derived oxygen, thereby creating a state of abnormal habitat filtering that lowers colonization resistance against *Enterobacterales* (155).

Notably, a loss of epithelial hypoxia weakens colonization resistance against *S. Typhimurium* even in the presence of normal concentrations of microbiota-derived short-chain fatty acids (89). These results appear to be at odds with *in vitro* findings that the presence of short-chain fatty acids inhibits growth of *Enterobacterales* in murine fecal homogenates and that oxygen alone is not sufficient to overcome this growth inhibition (108). A factor lacking in the *in vitro* experiments is a shift in epithelial energy metabolism, which is required to lower colonization resistance against an avirulent *S. Typhimurium* strain (i.e., a strain lacking both type III secretion systems [T3SSs]) in mice harboring normal levels of microbiota-derived short-chain fatty acids (89). These observations suggest that when cells derive energy through aerobic glycolysis (the conversion of glucose into lactate even in the presence of oxygen [156]), the epithelium releases factors in addition to oxygen that weaken colonization resistance against *S. Typhimurium*.

Metabolite profiling reveals that lactate is the most abundant metabolite in the gut lumen during *S. Typhimurium*-induced colitis, while only small amounts of this compound are detected in mock-infected mice (59). A similar increase in the luminal lactate concentration is also observed after antibiotic treatment, but this increase is blunted when mice are treated with a PPAR- $\gamma$  (peroxisome proliferator-activated receptor gamma) agonist that polarizes host cell metabolism toward oxidative phosphorylation, which is consistent with the idea that an increase in the luminal lactate concentration is mostly derived from a conversion of glucose into lactate by host cells (59). During its expansion in the gut microbiota, *S. Typhimurium* converts lactate into pyruvate using a NAD-independent lactate dehydrogenase (encoded by *lldD*), which transfers electrons from lactate to oxygen using cytochrome *bd* oxidase (encoded by *cydA*), thus linking lactate utilization in the gut to the presence of host-derived oxygen (59). Notably, *S. Enteritidis* can overcome growth inhibition by short-chain fatty acids *in vitro* when lactate is added to murine fecal homogenates (53), pointing to catabolism of host-derived lactate as a possible mechanism to overcome niche modification by microbiota-derived short-chain fatty acids. Finally, the terminal steps in acetate production through the phosphotransacetylase-acetate kinase (Pta-AckA) pathway are required for *S. Typhimurium* to overcome colonization resistance in chickens (157). Thus, it is tempting to speculate that epithelial release of lactate and oxygen cooperatively enables *S. Typhimurium* to ramp up intracellular acetate production to limit

diffusion of microbiota-derived acetic acid into the cytosol, thereby preventing disruption of pH homeostasis. However, additional work is needed to test this hypothesis.

Considering all of these studies suggests that an increased abundance of *Enterobacterales* is a microbial signature of dysbiosis that often involves abnormal habitat filtering by the host. During homeostasis, epithelial hypoxia limits the availability of respiratory electron acceptors (i.e., oxygen and nitrate), thereby filtering the habitat to ensure *Enterobacterales* remain minority species within the colonic microbiota. However, intestinal inflammation and/or a loss of epithelial hypoxia weaken colonization resistance by inducing a state of abnormal habitat filtering. In turn, abnormal habitat filtering leads to an elevated release of host-derived critical resources that enable commensal and pathogenic *Enterobacterales* to overcome niche modification by microbiota-derived short-chain fatty acids. The abundance of these limiting resources determines the abundance of *Enterobacterales* in the gut microbiota. In other words, limited availability of critical resources, such as respiratory electron acceptors and lactate, keeps a tight rein on *Enterobacterales*, which is responsible for the low abundance of this taxon during homeostasis. However, conditions that enlarge the availability of limiting resources drive dysbiosis characterized by an *Enterobacterales* expansion.

### **Niche Preemption by Endogenous *Enterobacterales***

**Competition with closely related species.** In addition to habitat filtering by the microbiota and the host, colonization resistance against *Salmonella* serovars also involves competition with closely related bacterial species that are resident in the gut microbiota. Commensal species within the *Enterobacterales* that are closely related genetically to *Salmonella* serovars (103) are a normal constituent in the fecal microbiota of humans (35) and other mammals (158). There are currently no approaches to specifically deplete *Enterobacterales* from the gut microbiota to ascertain their contribution to colonization resistance. However, not all laboratory mice harbor endogenous *Enterobacterales* (51), which is due to variability in animal husbandry practices between vendors. Many vendors of laboratory mice engraft germfree animals with altered Schaedler flora (159) to establish a baseline microbiota in their foundation breeding colonies, prior to transferring animals into barrier production, where microbiota assembly proceeds while animals are screened to prevent specific pathogens from entering the colony (specific-pathogen-free mice) (160). The screening procedures for specific pathogens differ between vendors, resulting in mice from some suppliers to remain *Enterobacterales*-free, while specific-pathogen-free procedures from others do not exclude commensal or opportunistic *Enterobacterales* from engrafting during microbiota assembly. Notably, comparison of genetically similar mice from different vendors reveals that the presence of endogenous *Enterobacterales*, which are minority species in the gut microbiota, results in a 100-fold increase in colonization resistance against *S. Typhimurium*, illustrating that competition with closely related species plays an important role in protecting against infection (51). Commensal *Enterobacterales*, such as *E. coli*, also enhance colonization resistance against *Salmonella* serovars in gnotobiotic mice (161), gnotobiotic piglets (162), or day-of-hatch chicks (50) or in a mouse model of high-fat diet (163).

**Keystone species limit the availability of critical resources.** Recent work is beginning to elucidate the mechanisms through which endogenous *Enterobacterales* contribute to colonization resistance against *Salmonella* serovars. Germ-free mice engrafted with defined microbial communities fail to confer colonization resistance against *S. Typhimurium* when pathways involved in microbial respiration are underrepresented compared to microbiota of conventional mice (164). However, colonization resistance can be strengthened by supplementing the defined microbial community with facultative anaerobic species, including *E. coli*, *Streptococcus danieliae*, and *Staphylococcus xylosum*, a correlation that points to the presence of respiratory pathways in the microbial community as a factor important for protection against *S. Typhimurium* infection (164). Interestingly, a commensal avian *E. coli* isolate competes more successfully with *S. Enteritidis* for oxygen when the commensal establishes gut colonization in neonatal



chicks prior to pathogen challenge, compared to when both species are introduced simultaneously (50). This finding suggests that endogenous *Enterobacteriales* have a competitive advantage over similar species that attempt to enter the ecosystem since priority effects provide them with access to growth-limiting resources. Although the precise mechanisms by which niche preemption enables endogenous *Enterobacteriales* to gain priority access to oxygen remain obscure, an intact aerobic metabolism (i.e., a functional cytochrome *bd* oxidase) is required for endogenous *E. coli* to confer colonization resistance against *Salmonella* serovars in mice (51). Thus, one of the mechanisms contributing to colonization resistance against *Salmonella* serovars is competition with endogenous *Enterobacteriales* for host-derived respiratory electron acceptors.

In addition to respiratory electron acceptors, *Enterobacteriales* compete for nutritional resources. For example, the concentrations of many monosaccharides become elevated in colon contents during antibiotic treatment (123), which supports growth of *S. Typhimurium* in the gut (165, 166). Some monosaccharides become oxidized (167) because an antibiotic-mediated depletion of short-chain fatty acids induces nitric oxide production by recruiting inflammatory monocytes to the colonic mucosa (168) and by increasing inducible nitric oxide synthase (iNOS) production in the colonic epithelium (89). Oxidation of monosaccharides by RNS in the gut lumen generates acidic sugars, such as glucarate and galactarate, which drive a postantibiotic expansion of *S. Typhimurium* (167). There is evidence to suggest that pathogen engraftment in the microbiota can be blocked through nutrient competition with endogenous *Enterobacteriales*. *Klebsiella michiganensis* is a commensal member of the *Enterobacteriales* that confers colonization resistance against *E. coli* in a mouse model (169). However, *K. michiganensis*-mediated colonization resistance against *E. coli* is lost when mice receive galactitol, a poorly absorbed sugar alcohol that reaches the colon, where it promotes growth of *E. coli* over *K. michiganensis*, because the latter cannot utilize this carbon source (169). These data suggest that antibiotic treatment generates an environment in which growth of *Enterobacteriales* is fueled by monosaccharide catabolism. Niche preemption mediated by endogenous *Enterobacteriales* likely involves competition for these critical resources with pathogens that attempt to enter the ecosystem.

In essence, although members of the *Enterobacteriales* are minority species within the gut microbiota that are often present at levels below the limit of detection by conventional microbiota profiling (51), they play a key role in conferring protection against facultative anaerobic pathogens, such as *Salmonella* serovars. Thus, endogenous *Enterobacteriales* have a disproportionately large effect on colonization resistance relative to their abundance within the microbial community, which renders them keystone species. Studies on the underlying mechanism reveal that endogenous *Enterobacteriales* contribute to colonization resistance through niche preemption, a process that involves competition with *Salmonella* serovars for critical resources, such as respiratory electron acceptors and monosaccharides.

## NICHE OPPORTUNITIES CREATED BY VIRULENCE FACTORS

### Ecosystem Engineering by Virulence Factors Licenses Pathogen Engraftment

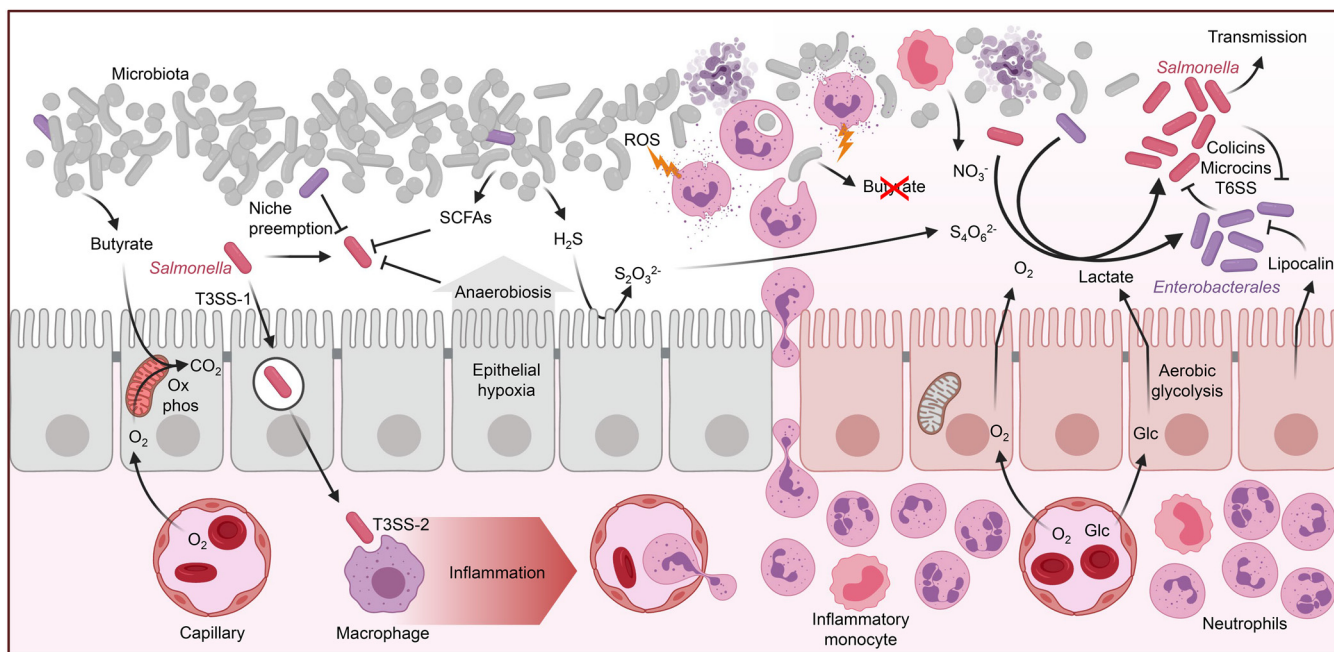
**Pathogens overcome colonization resistance—opportunists do not.** A mature gut microbiota is resistant to change (45, 56, 57) because the microbiome prevents engraftment of newly arriving commensal or opportunistic microbes through competition and habitat filtering (49). The phenomenon of microbiota resistance is testament to the fact that once the microbiota reaches a stable equilibrium state, priority effects pose an all-but-impenetrable barrier to engraftment of new commensal or opportunistic bacterial species belonging to the *Enterobacteriales*. For example, priority effects prevent replacement of resident endogenous *Enterobacteriales* present in the human fecal microbiota (35), with recently emerged opportunistic pathogens, such as carbapenem-resistant *Enterobacteriaceae* (CRE), thereby limiting community spread of CRE. Due to priority effects, the only way for opportunistic CRE to engraft in the gut microbiota is during microbiota assembly or after microbiota disruption (e.g., after antibiotic

therapy). Disruption of the microbiota with antibiotics can clear a niche (54) and provide an advantage for opportunistic CRE over antibiotic-sensitive competitors. As a result, an antibiotic-mediated disruption of the gut microbiota predisposes patients in the intensive care unit to developing carriage and nosocomial infection with CRE, which commonly includes strains of *Klebsiella pneumoniae* and *E. coli* (170–174). Due to weakened colonization resistance in individuals on broad-spectrum antibiotics, nosocomial CRE infections are readily transmitted between patients by hospital workers. In turn, a weakening of colonization resistance drives an expansion of CRE in the gut microbiota, which is a source of opportunistic bloodstream infections in immunocompromised patients (175, 176). Due to the lack of treatment options, 40% of CRE infections lead to death (177, 178), which makes these opportunistic pathogens one of the most urgent threats to public health worldwide (179).

A key difference between infection with opportunistic pathogens, such as CRE, and frank pathogens, such as *Salmonella* serovars, is that only the latter can overcome host defenses in individuals with an intact immune system. In other words, whereas CRE infection requires that colonization resistance is weakened by antibiotics, *Salmonella* serovars can engraft in individuals even when their microbiota-nourishing immunity is intact. In immunocompetent individuals, both CRE and *Salmonella* serovars initially enter an ecosystem that does not support their growth because the host and the microbiota limit critical resources through competition and habitat filtering. As a result, CRE numbers decline, resulting in an extinction of the opportunistic pathogen. Colonization resistance can also lead to an extinction of *Salmonella* serovars, particularly when the challenge dose is low (51) (Fig. 6). However, if the challenge dose is high enough to ensure the pathogen can deploy its virulence factors prior to becoming extinct, the initial decline in *S. Typhimurium* numbers is halted and followed by a marked expansion, resulting in pathogen engraftment in the gut ecosystem (118). *S. Typhimurium* virulence factors are long known to trigger disease in an immunocompetent host (180–182), a characteristic that distinguishes frank pathogens from opportunists, but the importance of virulence factors in overcoming colonization resistance has come to light only recently (7, 8). Importantly, virulence factors of *S. Typhimurium* weaken colonization resistance not by targeting the microbiota but by manipulating the physiology of host cells, thereby inducing a state of abnormal habitat filtering that opens new niche opportunities (183).

**Virulence factors carve out a new nutrient niche for the pathogen.** The main virulence factors of *S. Typhimurium* are two T3SSs that enable the pathogen to invade the epithelial lining (T3SS-1) (180) and survive in host tissue (T3SS-2) (181). Each T3SS injects several dozen proteins, called effectors, into the cytosol of epithelial cells (for T3SS-1) or macrophages (for T3SS-2) (184) to induce bacterial entry (185) or ensure the spread of bacteria in tissue (186), respectively. For a detailed discussion type III secreted effector proteins and their activity on host cell physiology, the reader is referred to a recent review article devoted to this subject (187).

The presence of bacteria in tissue induces sterilizing immunity by activating pathogen recognition receptors (188–193), thereby triggering innate immune responses that orchestrate severe acute intestinal inflammation (182, 194, 195). Detection of fecal leukocytes in salmonellosis patients illustrates that *Salmonella*-induced intestinal inflammation is accompanied by migration of phagocytes into the intestinal lumen (196), where these host cells contribute to the production of tetrathionate and nitrate as discussed above (10, 141) (Fig. 6). In addition, migration of neutrophils into the intestinal lumen leads to a depletion of *Clostridia* (197, 198), the main butyrate producers in the gut microbiota (116, 117), thereby reducing butyrate concentrations in colon contents (118). Since butyrate is an agonist of PPAR- $\gamma$ , a nuclear receptor that activates mitochondrial bioenergetics in the colonic epithelium, depletion of this short-chain fatty acid shifts the epithelial energy metabolism toward aerobic glycolysis, thereby increasing epithelial oxygenation and diffusion of oxygen into the intestinal lumen (89, 118).



**FIG 6** *S. Typhimurium* uses its virulence factors for ecosystem engineering. During homeostasis, conversion of microbiota-derived butyrate to carbon dioxide ( $\text{CO}_2$ ) through mitochondrial oxidative phosphorylation (Ox phos) results in high epithelial oxygen ( $\text{O}_2$ ) consumption, which maintains epithelial hypoxia. Epithelial cells detoxify microbiota-derived hydrogen sulfide ( $\text{H}_2\text{S}$ ) by conversion into thiosulfate ( $\text{S}_2\text{O}_3^{2-}$ ). Upon entry, *S. Typhimurium* uses its virulence factors to invade the intestinal epithelium (T3SS-1) and survive in macrophages in host tissue (T3SS-2). However, prior to the development of host responses, anaerobiosis and niche preemption by endogenous *Enterobacteriales* limit access of the luminal *S. Typhimurium* population to resources critical for overcoming growth inhibition by short-chain fatty acids (SCFAs). As a result, the luminal *S. Typhimurium* population decreases, which can lead to a pathogen extinction if the challenge dose is low. In the meantime, the virulence factor-mediated tissue invasion is detected by the innate immune system, which results in orchestration of an inflammatory response characterized by cellular infiltrates that are dominated by neutrophils. The inflammatory response eventually clears the subpopulation of the pathogen that resides in tissue, but it also induces migration of phagocytes into the intestinal lumen. Luminal phagocytes release reactive oxygen species (ROS) and reactive nitrogen species that generate host-derived electron acceptors, including tetrathionate ( $\text{S}_4\text{O}_6^{2-}$ ) and nitrate ( $\text{NO}_3^-$ ). Luminal neutrophils also deplete butyrate-producing *Clostridia* from the gut microbiota, which reduces mitochondrial bioenergetics in the intestinal epithelium. The consequent shift in epithelial energy metabolism to aerobic glycolysis, the conversion of glucose (Glc) into lactate, is associated with elevated epithelial release of oxygen and lactate. In turn, these changes in the luminal environment create a state of abnormal habitat filtering, thereby providing *S. Typhimurium* with critical resources (nitrate, tetrathionate, oxygen, and lactate) to expand in the gut microbiota, which is required for pathogen transmission by the fecal oral route. The new nutrient niche created by virulence factor-induced inflammation also supports growth of endogenous *Enterobacteriales*, provided they can overcome growth inhibition by lipocalin-2 (Lipocalin), an antimicrobial protein released by epithelial cells during intestinal inflammation. Through this chain of events, virulence factor-mediated ecosystem engineering creates a new nutrient niche in which *S. Typhimurium* and endogenous *Enterobacteriales* battle for supremacy using their antimicrobial weaponry, including colicins, microcins and type VI secretion systems (T6SS). (Created with BioRender.com.)

*Salmonella*-induced colitis also makes host-derived lactate the most abundant metabolite in the gut lumen (59).

Collectively, these virulence factor-induced changes in the gut environment trigger a state of abnormal habitat filtering, which is characterized by markedly elevated luminal concentrations of critical resources to support pathogen growth, including tetrathionate (10), nitrate (144, 145), oxygen (118), and lactate (59) (Fig. 6). These observations establish the concept that *S. Typhimurium* uses its virulence factors for ecosystem engineering, a process culminating in the generation of a new nutrient niche that supports pathogen engraftment into the gut ecosystem (94, 183). The consequent expansion of *S. Typhimurium* in the gut microbiota is required for pathogen transmission by the fecal-oral route (118, 199), which represents the principal driving force of natural selection for this strategy of ecosystem invasion.

**New niche opportunities create competition.** A drawback of ecosystem engineering is that the new nutrient niche generated by *S. Typhimurium* virulence factors can also be occupied by endogenous *Enterobacteriales*. Since *S. Typhimurium* and endogenous *Enterobacteriales* encounter the newly engineered nutrient niche simultaneously, presumably neither one gains an advantage through priority effects, which levels the playing field. During the fierce competition that ensues for niche occupancy,

contestants deploy antimicrobial weaponry to gain the upper hand in battling for critical resources. *S. Typhimurium*-induced intestinal inflammation increases concentrations of bile acids in the colon (200), a signal to induce expression of a type VI secretion system (T6SS), which is used by the pathogen to kill commensal competitors, such as *K. oxytoca* (201) (Fig. 6). One of the resources *S. Typhimurium* and endogenous *Enterobacterales* compete for is iron because the availability of this trace element is reduced in the inflamed gut. Reduced iron availability during inflammation requires bacteria to release small molecular weight ferric iron chelators, termed siderophores, to acquire this essential metal (202). The siderophore produced by most *Enterobacterales*, enterobactin, is neutralized by the host protein lipocalin-2 (203, 204), which is released into the gut lumen during intestinal inflammation (205, 206). *Salmonella* serovars adapt to this environment by producing a glycosylated derivative of enterobactin, termed salmochelin (207), which is not neutralized by lipocalin-2 (208), thus providing the pathogen with a growth advantage over competitors that rely solely on enterobactin for iron acquisition (209). However, the probiotic *E. coli* strain Nissle 1917 releases salmochelin derivatives conjugated to antimicrobial peptides, termed microcins M and H47 (210), which are internalized by salmochelin uptake systems of *Salmonella* serovars, thereby providing the commensal with a competitive advantage over the pathogen (211). The need to synthesize outer membrane siderophore receptors in the inflamed gut also provides an opportunity to battle related *Enterobacterales* by releasing colicins, which are bacteriocins with limited host range that commonly use siderophore receptors to enter their target cell (212). However, the T6SS is only induced when inflammation increases the concentration of bile acids (201), and neither microcins nor colicins provide a competitive advantage in the absence of intestinal inflammation because iron limitation generated by this host response induces expression of receptors for microcins and colicins in *Enterobacterales* (211, 212). Thus, *Enterobacterales* restrict the use of their antimicrobial weaponry to a state of abnormal habitat filtering.

All things considered, colonization resistance of mature microbial communities constitutes a formidable barrier that blocks an engraftment of commensal or opportunistic *Enterobacterales*. As a result, windows of opportunity for engrafting these species are limited to microbiota assembly in childhood or to episodes of weakened colonization resistance, which can be induced, for example, by antibiotics (Fig. 3A). In contrast, pathogenic *Enterobacterales*, such as *Salmonella* serovars, can overcome colonization resistance in immunocompetent individuals by using their virulence factors for ecosystem engineering (Fig. 6). The pathogen remodels the gut ecosystem by using its virulence factors to trigger intestinal inflammation. The consequent changes in the metabolite landscape create a state of abnormal habitat filtering that provides niche opportunities, which is a crucial determinant of the pathogen's success in ecosystem invasion. Importantly, this strategy for ecosystem invasion is limited to pathogens, because ecosystem engineering by virulence factors generates collateral damage, thereby producing signs of disease, the defining characteristic of pathogens. However, a drawback of this strategy for ecosystem invasion is that virulence factors engineer a nutrient niche that also accommodates related *Enterobacterales* species. As a result, the nutrient niche engineered by virulence factors of the pathogen provides a playground in which *S. Typhimurium* and endogenous *Enterobacterales* use their antimicrobial weaponry to fight for supremacy (94).

### Reconstructing *Salmonella's* Nutrient Niche from the Ruins

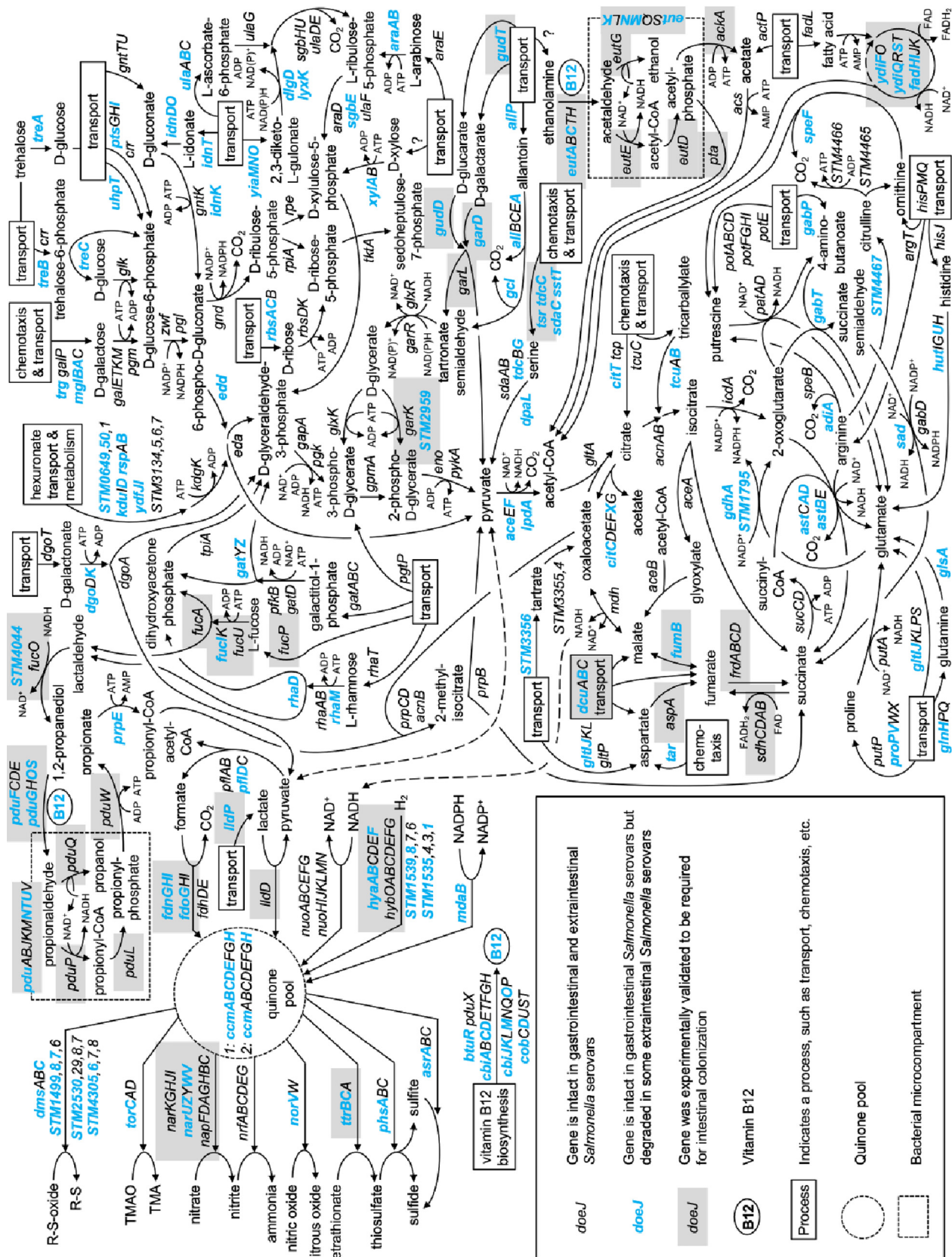
**Genome-guided assembly of a metabolic network for gut colonization.** Information on how intestinal inflammation alters the luminal habitat is key to understanding why this condition gives rise to imbalances in the gut microbiota that are linked to various noncommunicable diseases, such as IBD (152), colorectal cancer (77), or cardiovascular disease (78). Intestinal inflammation induced by *Salmonella* serovars can be used to model this state of abnormal habitat filtering, but our knowledge of the consequent changes in the luminal environment is still incomplete. The metabolic pathways the pathogen uses to fuel its growth in the inflamed gut can provide a window into the



nutrient niche *S. Typhimurium* occupies, which in turn offers clues about how inflammation alters the habitat of the gut microbiota (96). Notably, an experiment of nature makes it possible to identify these metabolic pathways through whole-genome comparison of *Salmonella* serovars (213–215).

Whereas the vast majority of *Salmonella* serovars are associated with gastroenteritis in humans, an infection that remains localized to the intestine and mesenteric lymph nodes, a few specialists have evolved to cause exclusively extraintestinal disease (e.g., *S. Typhi*) (216, 217). These specialists transmit from an extraintestinal reservoir (e.g., the gallbladder in case of *S. Typhi*) and no longer cause gastroenteritis in their respective hosts, thereby removing the driving force of natural selection for maintaining metabolic pathways required for growth in the inflamed gut (120). Consequently, genes that provide an adaptation to the nutrient niche gastrointestinal *Salmonella* serovars (e.g., *S. Typhimurium*) occupy in the inflamed intestine are dispensable in extraintestinal *Salmonella* serovars and are beginning to randomly degrade by point mutation. This ongoing experiment of nature explains the large numbers of degraded genes (pseudogenes) detected in the genomes of extraintestinal *Salmonella* serovars compared to gastrointestinal pathogens (218–222), a prominent genetic fingerprint that resembles an unsaturated mutagenesis of the pathways required for pathogen growth in the lumen of the inflamed gut (213, 223, 224). However, the emergence of extraintestinal *Salmonella* serovars is a relatively recent event linked to the Neolithic transition toward an agricultural and pastoralist economy (225, 226), suggesting that there was limited time for genome decay to leave its mark on their genomes. Since the process of genome degradation is quite incomplete, analysis of a single *Salmonella* serovar does not unveil a decaying metabolic network. Instead, whole-genome comparison of multiple extraintestinal and gastrointestinal *Salmonella* serovars is required to bring a network of genes to light that is degrading in genomes of extraintestinal pathogens but intact in genomes of gastrointestinal pathogens (Fig. 7) (213–215).

The metabolic network identified by such an *in silico* analysis contains more than 400 genes (213), only a fraction of which has yet been tested experimentally for their contribution to growth in an inflamed intestine (Fig. 7). The emerging experimental validation of these *in silico* predictions shows that genes for the import of host-derived lactate (*lldP*) and its cytochrome *bd* oxidase-dependent conversion into pyruvate (*lldD*), are required for luminal growth of *S. Typhimurium* during colitis (59). Pyruvate generated through this reaction can be converted by pyruvate formate lyase (encoded by *pflDC*) into acetyl coenzyme A (acetyl-CoA) and formate, two metabolites important for growth in the gut. The conversion of acetyl-CoA into acetate (*ackA pta*) is required for intestinal colonization of *S. Enteritidis* (157). Formate is degraded to carbon dioxide (CO<sub>2</sub>) and hydrogen (H<sub>2</sub>) by a nitrate respiration-dependent formate dehydrogenase (encoded by *fdnGHI*), which is required for growth of *E. coli* in the inflamed intestine (153). Hydrogen generated through this reaction supports growth of *S. Typhimurium* in the gut by serving as an electron donor (*hybOABCDEFG*) (227) for fumarate respiration (*frdABCD*), which is powered by exogenous aspartate or malate (*dcuABC*, *aspA*, and *fumB*) (124). Chemotaxis toward nitrate (encoded by *tsr*) (228), nitrate respiration (mediated by a periplasmic nitrate reductase encoded by *napFDAGHBC*) (145) and tetrathionate respiration (mediated by a tetrathionate reductase encoded by *ttrABC*) (10) are required for growth of *S. Typhimurium* in the niche it occupies in the inflamed intestine, in part because anaerobic respiration powers bacterial microcompartments that function in the catabolism of microbiota-derived fermentation products, including 1,2-propanediol (*pduABCDEFGHIJKLMNOQSTUVWX*) (229) and ethanolamine (*eutSPQTMNEJGHABCLKR*) (230). Nitrate respiration is also required for *S. Typhimurium* to catabolize microbiota-derived fermentation products, including succinate (231) (*sdhCDAB*) and butyrate (*ydiFO ydiQRST fadHIJK*) (232, 233). Finally, catabolism of some monosaccharides plays a role during *S. Typhimurium* gut colonization, as shown for fucose (*fucAO fucPIKUR*), glucarate (*gudDT*), and galactarate (*garDL STM2959*) (165, 167). Thus, the computer-generated concept that gene decay in extraintestinal *Salmonella*



**FIG 7** Reconstruction of a metabolic network required for growth in an engineered niche. Whole-genome comparison of 5 genomes representing extraintestinal *Salmonella* serovars and 10 genomes representing gastrointestinal *Salmonella* serovars reveals metabolic pathways/gastrointestinal pathogens use to fuel their growth in the inflamed gut (213). The graphic shows pathways that are degrading in (Continued on next page)

serovars defines a large metabolic network required for growth of gastrointestinal *Salmonella* serovars in the gut lumen (213–215) has been validated by numerous experimental studies (59, 124, 145, 153, 157, 167, 229, 231, 233). However, the majority of genes in this web still remain to be analyzed.

**Predicting the metabolic landscape in *Salmonella*'s nutrient niche.** Glancing at the hypothetical metabolic pathways identified by comparative genome analysis (213) provides a preview of the resources that might be available in the nutrient niche engineered by *S. Typhimurium* virulence factors. The metabolic network suggests that *S. Typhimurium* has access to numerous monosaccharides (including glucose, gluconate, galactose, galactonate, trehalose, rhamnose, ribose, xylose, arabinose, idonate, 2,3-diketo-gulonate, hexunonate, and galactitol) and amino acids (including serine, histidine, arginine, glutamate, aspartate, and proline) (Fig. 7), indicating its nutrient niche differs from the habitat of the noninflamed gut, where the microbiota depletes these critical resources (119, 165). It is also apparent from this model that the inflammatory host response might generate several respiratory electron acceptors in addition to nitrate (144), tetrathionate (10), and oxygen (118), which includes sulfite, thiosulfate, nitric oxide, nitrite, *S*-oxides ( $R_2-SO$ ), and *N*-oxides ( $R_3-N^+-O^-$ ), such as trimethylamine *N*-oxide (TMAO) (Fig. 7). Whereas nitric oxide is directly derived from inflammatory monocytes (141), *S*-oxides and *N*-oxides can be generated in the gut lumen when ROS and RNS diffuse away from host cells and react with organic sulfides and tertiary amines present in the intestinal lumen (234, 235). Furthermore, ROS and RNS released by luminal phagocytes react to form nitrate and tetrathionate, which are converted to nitrite and thiosulfate through nitrate respiration and tetrathionate respiration, respectively (10, 141, 147). Finally, sulfite is the product of thiosulfate respiration (236). Thus, the projected generation of an array of different respiratory electron acceptors in the gut lumen (Fig. 7) is expected to require recruitment of phagocytes into the intestinal lumen, which is a by-product of intestinal inflammation triggered by *S. Typhimurium* virulence factors (131).

In a nutshell, *in silico* analysis predicts that T3SS-1- and T3SS-2-mediated intestinal inflammation engineers a nutrient niche that is characterized by an increased availability of diverse repertoires of monosaccharides, amino acids, and respiratory electron acceptors (213). These sweeping changes in the luminal metabolite landscape are projected to create a state of abnormal habitat filtering to support pathogen engraftment and drive its expansion in the gut microbiota, which is required for transmission (118, 199). The latter provides the ultimate driving force of natural selection that maintains the metabolic network depicted in Fig. 7 in gastrointestinal *Salmonella* serovars (213).

## CONCLUDING REMARKS

### What *Salmonella* Serovars Teach Us about Dysbiosis

To summarize the findings discussed above, the host and its microbiota cooperate to execute a nonspecific host defense mechanism, termed colonization resistance, which prevents ecosystem invasion by opportunistic pathogens. Microbiota-derived short-chain fatty acids filter the environment to exclude bacteria lacking mechanisms to maintain pH homeostasis with the available resources. The resources used for maintenance of pH homeostasis in *Clostridia* and *Bacteroidia* remain to be described, but the dominance of these obligate anaerobic bacteria in the gut microbiota is testament to their ability to avert disruption of pH homeostasis by short-chain fatty acids. Facultative anaerobic *Enterobacterales*, on the other hand, require respiratory electron acceptors (such as oxygen and nitrate) and additional unidentified host-derived resources to overcome growth inhibition by short-chain fatty acids. Hence, the availability of

### FIG 7 Legend (Continued)

genomes of extraintestinal *Salmonella* serovars (genes in blue font) but are intact in genomes of gastrointestinal *Salmonella* serovars. The metabolic network predicted by this *in silico* analysis provides a window into the nutrient niche that is engineered by *S. Typhimurium* virulence factors in the gut. (Adapted from reference 213.)



these critical resources determines the abundance of *Enterobacterales* within the gut microbiota. During homeostasis, epithelial hypoxia severely limits the availability of respiratory electron acceptors, thereby relegating *Enterobacterales* to an existence as minority species within the gut microbiota (Fig. 2). Despite their low abundance, these minority species have a disproportionately large effect on colonization resistance against *Salmonella* serovars by limiting the pathogen's access to critical resources through priority effects, which identifies endogenous *Enterobacterales* as keystone species within the gut microbiota.

During homeostasis, engraftment of opportunistic pathogens, such as CRE, is efficiently blocked through niche modification by microbiota-derived short-chain fatty acids, habitat filtering by epithelial hypoxia, and niche preemption by endogenous *Enterobacterales*. However, frank pathogens, such as gastrointestinal *Salmonella* serovars, can use their virulence factors to overcome these defenses in an immunocompetent individual by disturbing host-mediated habitat filtering. Ecosystem invasion forces gastrointestinal *Salmonella* serovars to overcome growth inhibition by microbiota-derived short-chain fatty acids, an ecological problem demanding an increased availability of critical resources, such as respiratory electron acceptors, that are kept in short supply by host-mediated habitat filtering and are poorly accessible to the pathogen due to competition with endogenous *Enterobacterales*. Success in gut ecosystem invasion requires the pathogen to endure until its virulence factors generate inflammatory host responses that boost the luminal availability of these critical resources, an outcome that becomes more likely when the challenge dose is high. Framing the outcome of infection as an ecological problem highlights the importance of virulence factors in remodeling the gut ecosystem by triggering inflammation, a host response that ultimately creates a state of abnormal habitat filtering, thereby providing new niche opportunities for the pathogen. These considerations identify gastrointestinal *Salmonella* serovars as ecosystem engineers, a pathogenic strategy inevitably linked to disease (i.e., gastroenteritis).

*S. Typhimurium* virulence factors engineer a nutrient niche that also accommodates related *Enterobacterales* species (Fig. 6). Thus, the state of abnormal habitat filtering created by *S. Typhimurium* virulence factors might share features with noncommunicable diseases associated with an expansion of *Enterobacterales* in the fecal microbiota (37, 237). Notably, *S. Typhimurium* virulence factors induce this state of abnormal habitat filtering by targeting only the host (7, 10, 118, 144). Extrapolating this insight to noncommunicable diseases suggests that a dysbiotic *Enterobacterales* expansion in the fecal microbiota is secondary to an underlying defect in host-mediated habitat filtering (34, 36, 131). Recent studies using mouse models of IBD and colorectal cancer provide compelling experimental support for this concept (147, 148, 150, 152–154, 238). Thus, lessons learned from studying *S. Typhimurium* ecosystem invasion paved the way for developing a mechanistic understanding of factors driving a microbial signature of dysbiosis in the fecal microbiota, which is observed in a spectrum of noncommunicable diseases.

### Where Do We Go from Here?

**New strategies to rebalance the microbiota.** The finding that dysbiosis is linked to many human diseases has generated hopes that microbiome research will identify novel treatment strategies. Whereas targeting the microbes themselves with fecal microbiota transplants (239), probiotics (240), antibiotics (241), or precision editing of the microbiota (148, 238) shows promise in treating some conditions, great challenges remain to adapt these therapies to the broad spectrum of diseases associated with dysbiosis. By trailblazing the concept that an expansion of *Enterobacterales* in the fecal microbiota is a signature of dysbiosis that is triggered by an underlying defect in host-mediated habitat filtering (34, 36, 131), research on *S. Typhimurium* pathogenesis has created great prospects for identifying alternative treatment targets for remediating dysbiosis. Provided that dysbiosis results from abnormal habitat filtering by the host, it stands to reason that the microbiota can be rebalanced by normalizing host-mediated habitat filtering. A proof of concept for this therapeutic strategy comes from studies

on IBD. Environmental risk factors for IBD include a history of antibiotic usage and a Western-style high-fat diet (242–244). These environmental risk factors cooperate to reduce mitochondrial bioenergetics in the colonic epithelium, thereby increasing epithelial oxygenation in the murine colon (152). In turn, oxygen emanating from the epithelial surface drives an expansion of endogenous *Enterobacterales* in the fecal microbiota, which exacerbates pre-IBD (152). Treatment with an agonist of PPAR- $\gamma$ , a nuclear receptor in the colonic epithelium that activates mitochondrial bioenergetics, restores epithelial hypoxia, thereby blunting an *Enterobacterales* expansion in mice with pre-IBD (152) and in ulcerative colitis patients (245). Thus, host-derived habitat filters represent promising treatment targets for rebalancing the microbiota in a broad range of noncommunicable diseases linked to gut dysbiosis.

**Expanding the microbiome toolbox.** Studies on *S. Typhimurium* ecosystem invasion have provided first insights into how normal habitat filtering can be disrupted, but our understanding of host-derived habitat filters in the colon is still incomplete. Furthermore, identifying host-derived habitat filters that govern microbiota assembly at body surfaces other than the colon represents an immense task that remains to be achieved before we can hope to understand dysbiosis at these habitats. Following the example of *Salmonella* serovars, virulence factors of pathogens colonizing other surfaces, such as the respiratory tract or the reproductive tract, provide countless opportunities for identifying host-derived habitat filters at these sites. In turn, this information is expected to reveal what conditions contribute to normal habitat filtering at these body surfaces and how virulence factors induce a state of abnormal habitat filtering that enables pathogens to invade the respective ecosystem. Researchers in bacterial pathogenesis are well positioned to produce such mechanistic insights into how normal habitat filtering maintains homeostasis at various body surfaces. Input from the bacterial pathogenesis field will be needed to identify habitat filters because this information cannot be gleaned simply from cataloging bacterial species names. In turn, identification of habitat filters will aid in the interpretation of microbiota profiling data by linking microbial signatures of dysbiosis to the disruption of habitat filtering by virulence factors. As more information becomes available, it might become possible to read microbiota profiling data in ways similar to a blood test result. In the not so distant future, a microbial signature of dysbiosis at a given body surface might indicate an underlying defect in a specific host-derived habitat filter, which in turn might suggest a treatment aimed at normalizing that function. These prospects make the study of ecosystem invasion by mucosal pathogens one of the most exciting emerging areas in microbiome research.

## ACKNOWLEDGMENTS

A.W.L.R. was supported by Public Health Service grant AI060555. Work in R.M.T.'s laboratory was supported by Public Health Service grants AI143253, AI149632, AI112949, AI089078, and AI109799. Work in A.J.B.'s laboratory was supported by award 650976 from the Crohn's and Colitis Foundation of America and by Public Health Service grants AI044170, AI096528, AI112949, AI146432, and AI153069.

## REFERENCES

- Bordenave G. 2003. Louis Pasteur (1822–1895). *Microbes Infect* 5:553–560. [https://doi.org/10.1016/S1286-4579\(03\)00075-3](https://doi.org/10.1016/S1286-4579(03)00075-3).
- Koch R. 1882. Die Aetiologie der Tuberkulose. *Berliner Klin Wochenschr* 15:221–230.
- Roux EYA. 1888. Contribution à l'étude de la diphtérie. *Ann Inst Pasteur* 2:421–499.
- Neisser M, Shiga K. 1903. Ueber freie Rezeptoren von Typhus- und Dysenteriebazillen und über das Dysenterietoxin. *Dtsch Med Wochenschr* 29:61–62. <https://doi.org/10.1055/s-0028-1138255>.
- Conradi H. 1903. Über lösliche, durch aseptische Autolyse erhaltene Giftstoffe von Ruhr- und Typhus-Bazillen. *Dtsch Med Wochenschr* 29:26–28. <https://doi.org/10.1055/s-0028-1138228>.
- Bohnhoff M, Drake BL, Miller CP. 1954. Effect of streptomycin on susceptibility of intestinal tract to experimental *Salmonella* infection. *Proc Soc Exp Biol Med* 86:132–137. <https://doi.org/10.3181/00379727-86-21030>.
- Stecher B, Robbiani R, Walker AW, Westendorf AM, Barthel M, Kremer M, Chaffron S, Macpherson AJ, Buer J, Parkhill J, Dougan G, von Mering C, Hardt WD. 2007. *Salmonella enterica* serovar typhimurium exploits inflammation to compete with the intestinal microbiota. *PLoS Biol* 5:2177–2189. <https://doi.org/10.1371/journal.pbio.0050244>.
- Barman M, Unold D, Shifley K, Amir E, Hung K, Bos N, Salzman N. 2008. Enteric salmonellosis disrupts the microbial ecology of the murine gastrointestinal tract. *Infect Immun* 76:907–915. <https://doi.org/10.1128/IAI.01432-07>.
- Kamada N, Kim YG, Sham HP, Vallance BA, Puente JL, Martens EC, Nunez

- G. 2012. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. *Science* 336:1325–1329. <https://doi.org/10.1126/science.1222195>.
10. Winter SE, Thiennimitr P, Winter MG, Butler BP, Huseby DL, Crawford RW, Russell JM, Bevins CL, Adams LG, Tsois RM, Roth JR, Baumler AJ. 2010. Gut inflammation provides a respiratory electron acceptor for *Salmonella*. *Nature* 467:426–429. <https://doi.org/10.1038/nature09415>.
  11. Lopez CA, Miller BM, Rivera-Chávez F, Velazquez EM, Byndloss MX, Chávez-Arroyo A, Lokken KL, Tsois RM, Winter SE, Baumler AJ. 2016. Virulence factors enhance *Citrobacter rodentium* expansion through aerobic respiration. *Science* 353:1249–1253. <https://doi.org/10.1126/science.aag3042>.
  12. Kamdar K, Khakpour S, Chen J, Leone V, Brulc J, Mangatu T, Antonopoulos DA, Chang EB, Kahn SA, Kirschner BS, Young G, DePaolo RW. 2016. Genetic and metabolic signals during acute enteric bacterial infection alter the microbiota and drive progression to chronic inflammatory disease. *Cell Host Microbe* 19:21–31. <https://doi.org/10.1016/j.chom.2015.12.006>.
  13. Rivera-Chavez F, Mekalanos JJ. 2019. Cholera toxin promotes pathogen acquisition of host-derived nutrients. *Nature* 572:244–248. <https://doi.org/10.1038/s41586-019-1453-3>.
  14. Tipton L, Darcy JL, Hynson NA. 2019. A developing symbiosis: enabling cross-talk between ecologists and microbiome scientists. *Front Microbiol* 10:292. <https://doi.org/10.3389/fmicb.2019.00292>.
  15. Berg G, Rybakova D, Fischer D, Cernava T, Verges MC, Charles T, Chen X, Cocolin L, Eversole K, Corral GH, Kazou M, Kinkel L, Lange L, Lima N, Loy A, Macklin JA, Maguin E, Mauchline T, McClure R, Mitter B, Ryan M, Sarand I, Smidt H, Schelkle B, Roume H, Kiran GS, Selvin J, Souza RSC, van Overbeek L, Singh BK, Wagner M, Walsh A, Sessitsch A, Schlöter M. 2020. Microbiome definition re-visited: old concepts and new challenges. *Microbiome* 8:103. <https://doi.org/10.1186/s40168-020-00875-0>.
  16. Spiga L, Winter SE. 2019. Using enteric pathogens to probe the gut microbiota. *Trends Microbiol* 27:243–253. <https://doi.org/10.1016/j.tim.2018.11.007>.
  17. Pedron T, Nigro G, Sansonetti PJ. 2016. From homeostasis to pathology: decrypting microbe-host symbiotic signals in the intestinal crypt. *Philos Trans R Soc Lond B Biol Sci* 371. <https://doi.org/10.1098/rstb.2015.0500>.
  18. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, Jones TF, Fazil A, Hoekstra RM, International Collaboration on Enteric Disease 'Burden of Illness' Studies. 2010. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis* 50:882–889. <https://doi.org/10.1086/650733>.
  19. Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, Crump JA. 2015. Global burden of invasive nontyphoidal *Salmonella* disease, 2010(1). *Emerg Infect Dis* 21:941–949. <https://doi.org/10.3201/eid2106.140999>.
  20. Loeffler F. 1892. Ueber Epidemien unter den im hygienischen Institute zu Greifswald gehaltenen Mäusen und über die Bekämpfung der Feldmausplage. *Zentbl Bakteriol Parasitenkunde* 11:129–141.
  21. Tsois RM, Xavier MN, Santos RL, Baumler AJ. 2011. How to become a top model: impact of animal experimentation on human *Salmonella* disease research. *Infect Immun* 79:1806–1814. <https://doi.org/10.1128/IAI.01369-10>.
  22. Carter PB, Collins FM. 1974. The route of enteric infection in normal mice. *J Exp Med* 139:1189–1203. <https://doi.org/10.1084/jem.139.5.1189>.
  23. de Goffau MC, Lager S, Sovio U, Gaccioli F, Cook E, Peacock SJ, Parkhill J, Charnock-Jones DS, Smith GCS. 2019. Human placenta has no microbiome but can contain potential pathogens. *Nature* 572:329–334. <https://doi.org/10.1038/s41586-019-1628-y>.
  24. Cornwell WK, Schwilk LD, Ackerly DD. 2006. A trait-based test for habitat filtering: convex hull volume. *Ecology* 87:1465–1471. [https://doi.org/10.1890/0012-9658\(2006\)87\[1465:ATTFHF\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2006)87[1465:ATTFHF]2.0.CO;2).
  25. Walter J, Ley R. 2011. The human gut microbiome: ecology and recent evolutionary changes. *Annu Rev Microbiol* 65:411–429. <https://doi.org/10.1146/annurev-micro-090110-102830>.
  26. Davenport ER, Sanders JG, Song SJ, Amato KR, Clark AG, Knight R. 2017. The human microbiome in evolution. *BMC Biol* 15:127. <https://doi.org/10.1186/s12915-017-0454-7>.
  27. Tiffany CR, Baumler AJ. 2019. Dysbiosis: from fiction to function. *Am J Physiol Gastrointest Liver Physiol* 317:G602–G608. <https://doi.org/10.1152/ajpgi.00230.2019>.
  28. Litvak Y, Baumler AJ. 2019. Microbiota-nourishing immunity: a guide to understanding our microbial self. *Immunity* 51:214–224. <https://doi.org/10.1016/j.immuni.2019.08.003>.
  29. van Best N, Rolle-Kampczyk U, Schaap FG, Basic M, Olde Damink SWM, Bleich A, Savelkoul PHM, von Bergen M, Penders J, Hornef MW. 2020. Bile acids drive the newborn's gut microbiota maturation. *Nat Commun* 11:3692. <https://doi.org/10.1038/s41467-020-17183-8>.
  30. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505:559–563. <https://doi.org/10.1038/nature12820>.
  31. MacArthur R, Levins R. 1967. Limiting similarity convergence and divergence of coexisting species. *Am Nat* 101:377–385. <https://doi.org/10.1086/282505>.
  32. Keddy PA. 1992. Assembly and response rules: two goals for predictive community ecology. *J Veget Sci* 3:157–164. <https://doi.org/10.2307/3235676>.
  33. Zheng L, Kelly CJ, Colgan SP. 2015. Physiologic hypoxia and oxygen homeostasis in the healthy intestine. a review in the theme: cellular responses to hypoxia. *Am J Physiol Cell Physiol* 309:C350–C360. <https://doi.org/10.1152/ajpcell.00191.2015>.
  34. Litvak Y, Byndloss MX, Baumler AJ. 2018. Colonocyte metabolism shapes the gut microbiota. *Science* 362:eaat9076. <https://doi.org/10.1126/science.aat9076>.
  35. Human Microbiome Project Committee. 2012. Structure, function, and diversity of the healthy human microbiome. *Nature* 486:207–214. <https://doi.org/10.1038/nature11234>.
  36. Litvak Y, Byndloss MX, Tsois RM, Baumler AJ. 2017. Dysbiotic *Proteobacteria* expansion: a microbial signature of epithelial dysfunction. *Curr Opin Microbiol* 39:1–6. <https://doi.org/10.1016/j.mib.2017.07.003>.
  37. Shin NR, Whon TW, Bae JW. 2015. *Proteobacteria*: microbial signature of dysbiosis in gut microbiota. *Trends Biotechnol* 33:496–503. <https://doi.org/10.1016/j.tibtech.2015.06.011>.
  38. Garrido D, Dallas DC, Mills DA. 2013. Consumption of human milk glycoconjugates by infant-associated bifidobacteria: mechanisms and implications. *Microbiology (Reading)* 159:649–664. <https://doi.org/10.1099/mic.0.064113-0>.
  39. Sela DA, Chapman J, Adeuya A, Kim JH, Chen F, Whitehead TR, Lapidus A, Rokhsar DS, Lebrilla CB, German JB, Price NP, Richardson PM, Mills DA. 2008. The genome sequence of *Bifidobacterium longum* subsp. *infantis* reveals adaptations for milk utilization within the infant microbiome. *Proc Natl Acad Sci U S A* 105:18964–18969. <https://doi.org/10.1073/pnas.0809584105>.
  40. Sela DA, Mills DA. 2010. Nursing our microbiota: molecular linkages between bifidobacteria and milk oligosaccharides. *Trends Microbiol* 18:298–307. <https://doi.org/10.1016/j.tim.2010.03.008>.
  41. Shepherd ES, DeLoache WC, Pruss KM, Whitaker WR, Sonnenburg JL. 2018. An exclusive metabolic niche enables strain engraftment in the gut microbiota. *Nature* 557:434–438. <https://doi.org/10.1038/s41586-018-0092-4>.
  42. Sonnenburg JL, Xu J, Leip DD, Chen CH, Westover BP, Weatherford J, Buhler JD, Gordon JI. 2005. Glycan foraging *in vivo* by an intestine-adapted bacterial symbiont. *Science* 307:1955–1959. <https://doi.org/10.1126/science.1109051>.
  43. El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henricsson B. 2013. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol* 11:497–504. <https://doi.org/10.1038/nrmicro3050>.
  44. Mackie RI, Sghir A, Gaskins HR. 1999. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 69:1035S–1045S. <https://doi.org/10.1093/ajcn/69.5.1035s>.
  45. Sprockett D, Fukami T, Relman DA. 2018. Role of priority effects in the early-life assembly of the gut microbiota. *Nat Rev Gastroenterol Hepatol* 15:197–205. <https://doi.org/10.1038/nrgastro.2017.173>.
  46. Martinez I, Maldonado-Gomez MX, Gomes-Neto JC, Kittana H, Ding H, Schmaltz R, Joglekar P, Cardona RJ, Marsteller NL, Kembel SW, Benson AK, Peterson DA, Ramer-Tait AE, Walter J. 2018. Experimental evaluation of the importance of colonization history in early-life gut microbiota assembly. *Elife* 7:e36521. <https://doi.org/10.7554/eLife.36521>.
  47. Cornell HV, Lawton JH. 1992. Species interactions, local and regional processes, and limits to the richness of ecological communities: a theoretical perspective. *J Anim Ecol* 61:1–12. <https://doi.org/10.2307/5503>.
  48. Pinto-Sanchez NR, Crawford AJ, Wiens JJ. 2014. Using historical biogeography to test for community saturation. *Ecol Lett* 17:1077–1085. <https://doi.org/10.1111/ele.12310>.
  49. Fukami T. 2015. Historical contingency in community assembly: integrating niches, species pools, and priority effects. *Annu Rev Ecol Syst* 46:1–23. <https://doi.org/10.1146/annurev-ecolsys-110411-160340>.
  50. Litvak Y, Mon KKZ, Nguyen H, Chanthavixay G, Liou M, Velazquez EM, Kutter L, Alcantara MA, Byndloss MX, Tiffany CR, Walker GT, Faber F, Zhu

- Y, Bronner DN, Byndloss AJ, Tsois RM, Zhou H, Baumler AJ. 2019. Commensal *Enterobacteriaceae* protect against *Salmonella* colonization through oxygen competition. *Cell Host Microbe* 25:128–139 e5. <https://doi.org/10.1016/j.chom.2018.12.003>.
51. Velazquez EM, Nguyen H, Heasley KT, Saechao CH, Gil LM, Rogers AWL, Miller BM, Rolston MR, Lopez CA, Litvak Y, Liou MJ, Faber F, Bronner DN, Tiffany CR, Byndloss MX, Byndloss AJ, Baumler AJ. 2019. Endogenous *Enterobacteriaceae* underlie variation in susceptibility to *Salmonella* infection. *Nat Microbiol* 4:1057–1064. <https://doi.org/10.1038/s41564-019-0407-8>.
  52. Meynell GG, Subbaiah TV. 1963. Antibacterial mechanisms of the mouse gut. I. Kinetics of infection by *Salmonella* Typhimurium in normal and streptomycin-treated mice studied with abortive transductants. *Br J Exp Pathol* 44:197–208.
  53. Bohnhoff M, Miller CP, Martin WR. 1964. Resistance of the mouse's intestinal tract to experimental *Salmonella* infection. I. Factors responsible for its loss following streptomycin treatment. *J Exp Med* 120:817–828. <https://doi.org/10.1084/jem.120.5.817>.
  54. David LA, Materna AC, Friedman J, Campos-Baptista MI, Blackburn MC, Perrotta A, Erdman SE, Alm EJ. 2014. Host lifestyle affects human microbiota on daily timescales. *Genome Biol* 15:R89. <https://doi.org/10.1186/gb-2014-15-7-r89>.
  55. Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R, Heath AC, Leibel RL, Rosenbaum M, Gordon JI. 2013. The long-term stability of the human gut microbiota. *Science* 341:1237439. <https://doi.org/10.1126/science.1237439>.
  56. Sommer F, Anderson JM, Bharti R, Raes J, Rosenstiel P. 2017. The resilience of the intestinal microbiota influences health and disease. *Nat Rev Microbiol* 15:630–638. <https://doi.org/10.1038/nrmicro.2017.58>.
  57. Litvak Y, Baumler AJ. 2019. The founder hypothesis: a basis for microbiota resistance, diversity in taxa carriage, and colonization resistance against pathogens. *PLoS Pathog* 15:e1007563. <https://doi.org/10.1371/journal.ppat.1007563>.
  58. Tap J, Mondot S, Levenez F, Pelletier E, Caron C, Furet JP, Ugarte E, Munoz-Tamayo R, Paslier DL, Nalin R, Dore J, Leclerc M. 2009. Towards the human intestinal microbiota phylogenetic core. *Environ Microbiol* 11:2574–2584. <https://doi.org/10.1111/j.1462-2920.2009.01982.x>.
  59. Gillis CC, Hughes ER, Spiga L, Winter MG, Zhu W, Furtado de Carvalho T, Chanin RB, Behrendt CL, Hooper LV, Santos RL, Winter SE. 2018. Dysbiosis-associated change in host metabolism generates lactate to support *Salmonella* growth. *Cell Host Microbe* 23:54–64 e6. <https://doi.org/10.1016/j.chom.2017.11.006>.
  60. Relman DA. 2012. The human microbiome: ecosystem resilience and health. *Nutr Rev* 70(Suppl 1):S2–S9. <https://doi.org/10.1111/j.1753-4887.2012.00489.x>.
  61. Proctor L. 2019. Priorities for the next 10 years of human microbiome research. *Nature* 569:623–625. <https://doi.org/10.1038/d41586-019-01654-0>.
  62. Petersen C, Round JL. 2014. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol* 16:1024–1033. <https://doi.org/10.1111/cmi.12308>.
  63. Olesen SW, Alm EJ. 2016. Dysbiosis is not an answer. *Nat Microbiol* 1:16228. <https://doi.org/10.1038/nmicrobiol.2016.228>.
  64. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. 2005. Host-bacterial mutualism in the human intestine. *Science* 307:1915–1920. <https://doi.org/10.1126/science.1104816>.
  65. Byndloss MX, Baumler AJ. 2018. The germ-organ theory of non-communicable diseases. *Nat Rev Microbiol* 16:103–110. <https://doi.org/10.1038/nrmicro.2017.158>.
  66. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA, Fraser-Liggett CM, Nelson KE. 2006. Metagenomic analysis of the human distal gut microbiome. *Science* 312:1355–1359. <https://doi.org/10.1126/science.1124234>.
  67. van Duynhoven J, Vaughan EE, Jacobs DM, Kemperman RA, van Velzen EJ, Gross G, Roger LC, Possemiers S, Smilde AK, Dore J, Westerhuis JA, Van de Wiele T. 2011. Metabolic fate of polyphenols in the human superorganism. *Proc Natl Acad Sci U S A* 108(Suppl 1):4531–4538. <https://doi.org/10.1073/pnas.1000098107>.
  68. Stainier DY. 2005. No organ left behind: tales of gut development and evolution. *Science* 307:1902–1904. <https://doi.org/10.1126/science.1108709>.
  69. O'Hara AM, Shanahan F. 2006. The gut flora as a forgotten organ. *EMBO Rep* 7:688–693. <https://doi.org/10.1038/sj.embor.7400731>.
  70. McFall-Ngai M. 2007. Adaptive immunity: care for the community. *Nature* 445:153. <https://doi.org/10.1038/445153a>.
  71. Lee YK, Mazmanian SK. 2010. Has the microbiota played a critical role in the evolution of the adaptive immune system? *Science* 330:1768–1773. <https://doi.org/10.1126/science.1195568>.
  72. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, Glickman JN, Siebert R, Baron RM, Kasper DL, Blumberg RS. 2012. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 336:489–493. <https://doi.org/10.1126/science.1219328>.
  73. Hooper LV, Littman DR, Macpherson AJ. 2012. Interactions between the microbiota and the immune system. *Science* 336:1268–1273. <https://doi.org/10.1126/science.1223490>.
  74. Gallo RL, Hultsch T, Farnaes L. 2016. Recognizing that the microbiome is part of the human immune system will advance treatment of both cancer and infections. *J Am Acad Dermatol* 74:772–774. <https://doi.org/10.1016/j.jaad.2015.11.039>.
  75. Marchesi JR, Adams DH, Fava F, Hermes GD, Hirschfield GM, Hold G, Quraishi MN, Kinross J, Smidt H, Tuohy KM, Thomas LV, Zoetendal EG, Hart A. 2016. The gut microbiota and host health: a new clinical frontier. *Gut* 65:330–339. <https://doi.org/10.1136/gutjnl-2015-309990>.
  76. Khlystova ZS. 1976. Lymph-node and spleen morphology in gnotobiotic rats. *Bull Exp Biol Med* 81:770–772. <https://doi.org/10.1007/BF00797161>.
  77. Arthur JC, Perez-Chanona E, Muhlbauer M, Tomkovich S, Uronis JM, Fan TJ, Campbell BJ, Abujamel T, Dogan B, Rogers AB, Rhodes JM, Stintzi A, Simpson KW, Hansen JJ, Keku TO, Fodor AA, Jobin C. 2012. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science* 338:120–123. <https://doi.org/10.1126/science.1224820>.
  78. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 472:57–63. <https://doi.org/10.1038/nature09922>.
  79. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngombu C, Blanchard C, Junt T, Nicod LP, Harris NL, Marsland BJ. 2014. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med* 20:159–166. <https://doi.org/10.1038/nm.3444>.
  80. Arrieta MC, Finlay BB. 2012. The commensal microbiota drives immune homeostasis. *Front Immunol* 3:33. <https://doi.org/10.3389/fimmu.2012.00033>.
  81. Al-Asmakh M, Zadjali F. 2015. Use of germ-free animal models in microbiota-related research. *J Microbiol Biotechnol* 25:1583–1588. <https://doi.org/10.4014/jmb.1501.01039>.
  82. Bocci V. 1992. The neglected organ: bacterial flora has a crucial immunostimulatory role. *Perspect Biol Med* 35:251–260. <https://doi.org/10.1353/pbm.1992.0004>.
  83. Dethlefsen L, McFall-Ngai M, Relman DA. 2007. An ecological and evolutionary perspective on human-microbe mutualism and disease. *Nature* 449:811–818. <https://doi.org/10.1038/nature06245>.
  84. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, Shilo S, Lador D, Vila AV, Zmora N, Pevsner-Fischer M, Israel D, Kosower N, Malka G, Wolf BC, Avnit-Sagi T, Lotan-Pompan M, Weinberger A, Halpern Z, Carmi S, Fu J, Wijnmenga C, Zernakova A, Elinav E, Segal E. 2018. Environment dominates over host genetics in shaping human gut microbiota. *Nature* 555:210–215. <https://doi.org/10.1038/nature25973>.
  85. Komai M, Shirakawa H, Kimura S. 1988. Newly developed model for vitamin K deficiency in germfree mice. *Int J Vitamin Nutr Res* 58:55–59.
  86. Janeway CA, Jr, Medzhitov R. 2002. Innate immune recognition. *Annu Rev Immunol* 20:197–216. <https://doi.org/10.1146/annurev.immunol.20.083001.084359>.
  87. Kisseleva EP. 2014. Innate immunity underlies symbiotic relationships. *Biochemistry (Mosc)* 79:1273–1285. <https://doi.org/10.1134/S0006297914120013>.
  88. Byndloss MX, Litvak Y, Baumler AJ. 2019. Microbiota-nourishing immunity and its relevance for ulcerative colitis. *Inflamm Bowel Dis* 25:811–815. <https://doi.org/10.1093/ibd/izz004>.
  89. Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL, Torres TP, Byndloss AJ, Faber F, Gao Y, Litvak Y, Lopez CA, Xu G, Napoli E, Giulivi C, Tsois RM, Revzin A, Lebrilla CB, Bäuml AJ. 2017. Microbiota-activated PPAR-γ signaling inhibits dysbiotic *Enterobacteriaceae* expansion. *Science* 357:570–575. <https://doi.org/10.1126/science.aam9949>.
  90. Nadjsonbati MS, McGinty JW, Lyons-Cohen MR, Jaffe JB, DiPeso L, Schneider C, Miller CN, Pollack JL, Nagana Gowda GA, Fontana MF, Erle DJ, Anderson MS, Locksley RM, Raftery D, von Moltke J. 2018. Detection of succinate by intestinal Tuft cells triggers a type 2 innate immune circuit. *Immunity* 49:33–41. <https://doi.org/10.1016/j.immuni.2018.06.016>.



91. Morita N, Umemoto E, Fujita S, Hayashi A, Kikuta J, Kimura I, Haneda T, Imai T, Inoue A, Mimuro H, Maeda Y, Kayama H, Okumura R, Aoki J, Okada N, Kida T, Ishii M, Nabeshima R, Takeda K. 2019. GPR31-dependent dendrite protrusion of intestinal CX3CR1<sup>+</sup> cells by bacterial metabolites. *Nature* 566:110–114. <https://doi.org/10.1038/s41586-019-0884-1>.
92. Levy M, Blacher E, Elinav E. 2017. Microbiome, metabolites, and host immunity. *Curr Opin Microbiol* 35:8–15. <https://doi.org/10.1016/j.mib.2016.10.003>.
93. Levy M, Thaiss CA, Elinav E. 2016. Metabolites: messengers between the microbiota and the immune system. *Genes Dev* 30:1589–1597. <https://doi.org/10.1101/gad.284091.116>.
94. Tsois RM, Bauml A. 2020. Gastrointestinal host-pathogen interaction in the age of microbiome research. *Curr Opin Microbiol* 53:78–89. <https://doi.org/10.1016/j.mib.2020.03.002>.
95. Liao MK. 2011. The lure of bacterial genetics: a tribute to John Roth. *J Microbiol Biol Educat* 12:85. <https://doi.org/10.1128/jmbe.v12i1.260>.
96. Rivera-Chavez F, Bauml A. 2015. The pyromaniac inside you: *Salmonella* metabolism in the host gut. *Annu Rev Microbiol* 69:31–48. <https://doi.org/10.1146/annurev-micro-091014-104108>.
97. Nicolaou KC, Rigol S. 2018. A brief history of antibiotics and select advances in their synthesis. *J Antibiot (Tokyo)* 71:153–184. <https://doi.org/10.1038/ja.2017.62>.
98. Keefer CS. 1951. Alterations in normal bacterial flora of man and secondary infections during antibiotic therapy. *Am J Med* 11:665–666. [https://doi.org/10.1016/0002-9343\(51\)90017-4](https://doi.org/10.1016/0002-9343(51)90017-4).
99. Smith MH, Lossli CG. 1952. Hospital cross-infections and their control. *J Pediatr* 41:844–852. [https://doi.org/10.1016/s0022-3476\(52\)80304-x](https://doi.org/10.1016/s0022-3476(52)80304-x).
100. Smith DT. 1952. The disturbance of the normal bacterial ecology by the administration of antibiotics with the development of new clinical syndromes. *Ann Intern Med* 37:1135–1143. <https://doi.org/10.7326/0003-4819-37-6-1135>.
101. Meynell GG. 1963. Antibacterial mechanisms of the mouse gut. II. The role of Eh and volatile fatty acids in the normal gut. *Br J Exp Pathol* 44:209–219.
102. Miller CP, Bohnhoff M. 1963. Changes in the mouse's enteric microflora associated with enhanced susceptibility to *Salmonella* infection following streptomycin treatment. *J Infect Dis* 113:59–66. <https://doi.org/10.1093/infdis/113.1.59>.
103. Adeolu M, Alnajr S, Naushad S, S Gupta R. 2016. Genome-based phylogeny and taxonomy of the '*Enterobacteriales*': proposal for *Enterobacteriales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaceae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. *Int J Syst Evol Microbiol* 66:5575–5599. <https://doi.org/10.1099/ijsem.0.001485>.
104. Russell JB, Diez-Gonzalez F. 1998. The effects of fermentation acids on bacterial growth. *Adv Microb Physiol* 39:205–234. [https://doi.org/10.1016/s0065-2911\(08\)60017-x](https://doi.org/10.1016/s0065-2911(08)60017-x).
105. Freter R, Brickner H, Botney M, Cleven D, Aranki A. 1983. Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora. *Infect Immun* 39:676–685. <https://doi.org/10.1128/IAI.39.2.676-685.1983>.
106. Freter R, Brickner H, Fekete J, Vickerman MM, Carey KE. 1983. Survival and implantation of *Escherichia coli* in the intestinal tract. *Infect Immun* 39:686–703. <https://doi.org/10.1128/IAI.39.2.686-703.1983>.
107. Jacobson A, Lam L, Rajendram M, Tamburini F, Honeycutt J, Pham T, Van Treuren W, Pruss K, Stabler SR, Lugo K, Bouley DM, Vilches-Moure JG, Smith M, Sonnenburg JL, Bhatt AS, Huang KC, Monack D. 2018. A gut commensal-produced metabolite mediates colonization resistance to *Salmonella* infection. *Cell Host Microbe* 24:296–307 e7. <https://doi.org/10.1016/j.chom.2018.07.002>.
108. Sorbara MT, Dubin K, Littmann ER, Moody TU, Fontana E, Seok R, Leiner IM, Taur Y, Peled JU, van den Brink MRM, Litvak Y, Bauml AJ, Chaubard JL, Pickard AJ, Cross JR, Pamer EG. 2019. Inhibiting antibiotic-resistant *Enterobacteriaceae* by microbiota-mediated intracellular acidification. *J Exp Med* 216:84–98. <https://doi.org/10.1084/jem.20181639>.
109. Osbelt L, Thiemann S, Smit N, Lesker TR, Schroter M, Galvez EJC, Schmidt-Hohagen K, Pils MC, Muhlen S, Dersch P, Hiller K, Schluter D, Neumann-Schaal M, Strowig T. 2020. Variations in microbiota composition of laboratory mice influence *Citrobacter rodentium* infection via variable short-chain fatty acid production. *PLoS Pathog* 16:e1008448. <https://doi.org/10.1371/journal.ppat.1008448>.
110. Salmond CV, Kroll RG, Booth IR. 1984. The effect of food preservatives on pH homeostasis in *Escherichia coli*. *J Gen Microbiol* 130:2845–2850. <https://doi.org/10.1099/00221287-130-11-2845>.
111. Slonczewski JL, Rosen BP, Alger JR, Macnab RM. 1981. pH homeostasis in *Escherichia coli*: measurement by <sup>31</sup>P nuclear magnetic resonance of methylphosphonate and phosphate. *Proc Natl Acad Sci U S A* 78:6271–6275. <https://doi.org/10.1073/pnas.78.10.6271>.
112. Zilberstein D, Agmon V, Schuldiner S, Padan E. 1984. *Escherichia coli* intracellular pH, membrane potential, and cell growth. *J Bacteriol* 158:246–252. <https://doi.org/10.1128/JB.158.1.246-252.1984>.
113. Chakraborty S, Liu L, Fitzsimmons L, Porwollik S, Kim JS, Desai P, McClelland M, Vazquez-Torres A. 2020. Glycolytic reprogramming in *Salmonella* counters NOX2-mediated dissipation of ΔpH. *Nat Commun* 11:1783. <https://doi.org/10.1038/s41467-020-15604-2>.
114. Bohnhoff M, Miller CP, Martin WR. 1964. Resistance of the mouse's intestinal tract to experimental *Salmonella* infection. I. Factors which interfere with the initiation of infection by oral inoculation. *J Exp Med* 120:805–816. <https://doi.org/10.1084/jem.120.5.805>.
115. Roe AJ, McLaggan D, Davidson I, O'Byrne C, Booth IR. 1998. Perturbation of anion balance during inhibition of growth of *Escherichia coli* by weak acids. *J Bacteriol* 180:767–772. <https://doi.org/10.1128/JB.180.4.767-772.1998>.
116. Louis P, Flint HJ. 2009. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. *FEMS Microbiol Lett* 294:1–8. <https://doi.org/10.1111/j.1574-6968.2009.01514.x>.
117. Vital M, Howe AC, Tiedje JM. 2014. Revealing the bacterial butyrate synthesis pathways by analyzing (meta)genomic data. *mBio* 5:e00889-14. <https://doi.org/10.1128/mBio.00889-14>.
118. Rivera-Chavez F, Zhang LF, Faber F, Lopez CA, Byndloss MX, Olsan EE, Xu G, Velazquez EM, Lebrilla CB, Winter SE, Bauml AJ. 2016. Depletion of butyrate-producing clostridia from the gut microbiota drives an aerobic luminal expansion of *Salmonella*. *Cell Host Microbe* 19:443–454. <https://doi.org/10.1016/j.chom.2016.03.004>.
119. Caballero-Flores G, Pickard JM, Fukuda S, Inohara N, Nunez G. 2020. An enteric pathogen subverts colonization resistance by evading competition for amino acids in the gut. *Cell Host Microbe* <https://doi.org/10.1016/j.chom.2020.06.018>.
120. Hiyoshi H, Tiffany CR, Bronner DN, Bauml AJ. 2018. Typhoidal *Salmonella* serovars: ecological opportunity and the evolution of a new pathovar. *FEMS Microbiol Rev* 42:527–541. <https://doi.org/10.1093/femsre/fuy024>.
121. Stokes JL, Bayne HG. 1958. Growth-factor-dependent strains of salmonellae. *J Bacteriol* 76:417–421. <https://doi.org/10.1128/JB.76.4.417-421.1958>.
122. Virgilio R, Cordano AM. 1981. Naturally occurring prototrophic strains of *Salmonella* Typhi. *Can J Microbiol* 27:1272–1275. <https://doi.org/10.1139/m81-195>.
123. Theriot CM, Koenigsnecht MJ, Carlson PE, Jr, Hatton GE, Nelson AM, Li B, Huffnagle GB, J ZL, Young VB. 2014. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *Clostridium difficile* infection. *Nat Commun* 5:3114. <https://doi.org/10.1038/ncomms4114>.
124. Nguyen BD, Cuenca VM, Hartl J, Gul E, Bauer R, Meile S, Ruthi J, Margot C, Heeb L, Besser F, Escriva PP, Fetz C, Furter M, Laganenka L, Keller P, Fuchs L, Christen M, Porwollik S, McClelland M, Vorholt JA, Sauer U, Sunagawa S, Christen B, Hardt WD. 2020. Import of aspartate and malate by DcuABC drives H<sub>2</sub>/fumarate respiration to promote initial *Salmonella* gut-lumen colonization in mice. *Cell Host Microbe* 27:922–936 e6. <https://doi.org/10.1016/j.chom.2020.04.013>.
125. Vollaard EJ, Clasener HA, Janssen AJ. 1992. Co-trimoxazole impairs colonization resistance in healthy volunteers. *J Antimicrob Chemother* 30:685–691. <https://doi.org/10.1093/jac/30.5.685>.
126. Sorbara MT, Pamer EG. 2019. Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them. *Mucosal Immunol* 12:1–9. <https://doi.org/10.1038/s41385-018-0053-0>.
127. Ducarmon QR, Zwittink RD, Hornung BVH, van Schaik W, Young VB, Kuijper EJ. 2019. Gut microbiota and colonization resistance against. *Microbiol Mol Biol Rev* 83:e00007-19. <https://doi.org/10.1128/MMBR.00007-19>.
128. Pickard JM, Nunez G. 2019. Pathogen colonization resistance in the gut and its manipulation for improved health. *Am J Pathol* 189:1300–1310. <https://doi.org/10.1016/j.ajpath.2019.03.003>.
129. Libertucci J, Young VB. 2019. The role of the microbiota in infectious diseases. *Nat Microbiol* 4:35–45. <https://doi.org/10.1038/s41564-018-0278-4>.
130. Iacob S, Iacob DG, Luminos LM. 2018. Intestinal microbiota as a host defense mechanism to infectious threats. *Front Microbiol* 9:3328. <https://doi.org/10.3389/fmicb.2018.03328>.
131. Winter SE, Lopez CA, Bauml AJ. 2013. The dynamics of gut-associated

- microbial communities during inflammation. *EMBO Rep* 14:319–327. <https://doi.org/10.1038/embor.2013.27>.
132. Dubinkina VB, Tyakht AV, Odintsova VY, Yarygin KS, Kovarsky BA, Pavlenko AV, Ischenko DS, Popenko AS, Alexeev DG, Taraskina AY, Nasyrova RF, Krupitsky EM, Shalikiani NV, Bakulin IG, Shcherbakov PL, Skorodumova LO, Larin AK, Kostyukova ES, Abdulkhakov RA, Abdulkhakov SR, Malanin SY, Ismagilova RK, Grigoryeva TV, Iliina EN, Govorun VM. 2017. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome* 5:141. <https://doi.org/10.1186/s40168-017-0359-2>.
  133. Wang Z, Wang Q, Wang X, Zhu L, Chen J, Zhang B, Chen Y, Yuan Z. 2019. Gut microbial dysbiosis is associated with development and progression of radiation enteritis during pelvic radiotherapy. *J Cell Mol Med* 23:3747–3756. <https://doi.org/10.1111/jcmm.14289>.
  134. Pham TA, Clare S, Goulding D, Arasteh JM, Stares MD, Browne HP, Keane JA, Page AJ, Kumasaka N, Kane L, Mottram L, Harcourt K, Hale C, Arends MJ, Gaffney DJ, Sanger Mouse Genetics P, Dougan G, Lawley TD, Sanger Mouse Genetics Project. 2014. Epithelial IL-22RA1-mediated fucosylation promotes intestinal colonization resistance to an opportunistic pathogen. *Cell Host Microbe* 16:504–516. <https://doi.org/10.1016/j.chom.2014.08.017>.
  135. Mueller S, Saunier K, Hanisch C, Norin E, Alm L, Midtvedt T, Cresci A, Silvi S, Orpianesi C, Verdenelli MC, Clavel T, Koebnick C, Zunft HJ, Dore J, Blaut M. 2006. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl Environ Microbiol* 72:1027–1033. <https://doi.org/10.1128/AEM.72.2.1027-1033.2006>.
  136. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 104:13780–13785. <https://doi.org/10.1073/pnas.0706625104>.
  137. Normann E, Fahlen A, Engstrand L, Lilja HE. 2013. Intestinal microbial profiles in extremely preterm infants with and without necrotizing enterocolitis. *Acta Paediatr* 102:129–136. <https://doi.org/10.1111/apa.12059>.
  138. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, Hernandez RD, Lederman MM, Huang Y, Somsouk M, Deeks SG, Hunt PW, Lynch SV, McCune JM. 2013. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. *Sci Transl Med* 5:193ra91. <https://doi.org/10.1126/scitranslmed.3006438>.
  139. Fredricks DN. 2019. The gut microbiota and graft-versus-host disease. *J Clin Invest* 129:1808–1817. <https://doi.org/10.1172/JCI125797>.
  140. Braun T, Di Segni A, BenShoshan M, Asaf R, Squires JE, Farage Barhom S, Glick Saar E, Cesarkas K, Smollan G, Weiss B, Amit S, Keller N, Haberman Y. 2017. Fecal microbial characterization of hospitalized patients with suspected infectious diarrhea shows significant dysbiosis. *Sci Rep* 7:1088. <https://doi.org/10.1038/s41598-017-01217-1>.
  141. McLaughlin PA, Bettke JA, Tam JW, Leeds J, Bliska JB, Butler BP, van der Velden AWM. 2019. Inflammatory monocytes provide a niche for *Salmonella* expansion in the lumen of the inflamed intestine. *PLoS Pathog* 15:e1007847. <https://doi.org/10.1371/journal.ppat.1007847>.
  142. Loetscher Y, Wieser A, Lengefeld J, Kaiser P, Schubert S, Heikenwalder M, Hardt WD, Stecher B. 2012. *Salmonella* transiently reside in luminal neutrophils in the inflamed gut. *PLoS One* 7:e34812. <https://doi.org/10.1371/journal.pone.0034812>.
  143. Vazquez-Torres A, Jones-Carson J, Mastroeni P, Ischiropoulos H, Fang FC. 2000. Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric oxide synthase in experimental salmonellosis. I. Effects on microbial killing by activated peritoneal macrophages *in vitro*. *J Exp Med* 192:227–236. <https://doi.org/10.1084/jem.192.2.227>.
  144. Lopez CA, Winter SE, Rivera-Chavez F, Xavier MN, Poon V, Nuccio SP, Tsolis RM, Bauml AJ. 2012. Phage-mediated acquisition of a type III secreted effector protein boosts growth of salmonella by nitrate respiration. *mBio* 3:e00143-12. <https://doi.org/10.1128/mBio.00143-12>.
  145. Lopez CA, Rivera-Chavez F, Byndloss MX, Bauml AJ. 2015. The periplasmic nitrate reductase NapABC supports luminal growth of *Salmonella enterica* serovar Typhimurium during colitis. *Infect Immun* 83:3470–3478. <https://doi.org/10.1128/IAI.00351-15>.
  146. Wang S, El-Fahmawi A, Christian DA, Fang Q, Radaelli E, Chen L, Sullivan MC, Mistic AM, Ellringer JA, Zhu XQ, Winter SE, Hunter CA, Beiting DP. 2019. Infection-induced intestinal dysbiosis is mediated by macrophage activation and nitrate production. *mBio* 10:e00935-19. <https://doi.org/10.1128/mBio.00935-19>.
  147. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, Laughlin RC, Gomez G, Wu J, Lawhon SD, Popova IE, Parikh SJ, Adams LG, Tsolis RM, Stewart VJ, Bauml AJ. 2013. Host-derived nitrate boosts growth of *Escherichia coli* in the inflamed gut. *Science* 339:708–711. <https://doi.org/10.1126/science.1232467>.
  148. Zhu W, Winter MG, Byndloss MX, Spiga L, Duerkop BA, Hughes ER, Buttner L, de Lima Romao E, Behrendt CL, Lopez CA, Sifuentes-Dominguez L, Huff-Hardy K, Wilson RP, Gillis CC, Tukul C, Koh AY, Burstein E, Hooper LV, Bauml AJ, Winter SE. 2018. Precision editing of the gut microbiota ameliorates colitis. *Nature* 553:208–211. <https://doi.org/10.1038/nature25172>.
  149. Potgens SA, Brossel H, Sboarina M, Catry E, Cani PD, Neyrinck AM, Delzenne NM, Bindels LB. 2018. *Klebsiella oxytoca* expands in cancer cachexia and acts as a gut pathobiont contributing to intestinal dysfunction. *Sci Rep* 8:12321. <https://doi.org/10.1038/s41598-018-30569-5>.
  150. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, Finlay BB. 2007. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of *Enterobacteriaceae*. *Cell Host Microbe* 2:204. <https://doi.org/10.1016/j.chom.2007.08.002>.
  151. Carson D, Barry R, Hopkins EGD, Roumeliotis TI, Garcia-Weber D, Mullineaux-Sanders C, Elinav E, Arriemerlou C, Choudhary JS, Frankel G. 2019. *Citrobacter rodentium* induces rapid and unique metabolic and inflammatory responses in mice suffering from severe disease. *Cell Microbiol* 22:e13126. <https://doi.org/10.1111/cmi.13126>.
  152. Lee JY, Cevallos SA, Byndloss MX, Tiffany CR, Olsan EE, Butler BP, Young BC, Rogers AWL, Nguyen H, Kim K, Choi SW, Bae E, Lee JH, Min UG, Lee DM, Bauml AJ. 2020. High-fat diet and antibiotics cooperatively impair mitochondrial bioenergetics to trigger dysbiosis that exacerbates pre-inflammatory bowel disease. *Cell Host Microbe* 28:273–284. <https://doi.org/10.1016/j.chom.2020.06.001>.
  153. Hughes ER, Winter MG, Duerkop BA, Spiga L, Furtado de Carvalho T, Zhu W, Gillis CC, Buttner L, Smoot MP, Behrendt CL, Cherry S, Santos RL, Hooper LV, Winter SE. 2017. Microbial respiration and formate oxidation as metabolic signatures of inflammation-associated dysbiosis. *Cell Host Microbe* 21:208–219. <https://doi.org/10.1016/j.chom.2017.01.005>.
  154. Cevallos SA, Lee JY, Tiffany CR, Byndloss AJ, Johnston L, Byndloss MX, Bauml AJ. 2019. Increased epithelial oxygenation links colitis to an expansion of tumorigenic bacteria. *mBio* 10:e02244-19. <https://doi.org/10.1128/mBio.02244-19>.
  155. Rivera-Chavez F, Lopez CA, Bauml AJ. 2017. Oxygen as a driver of gut dysbiosis. *Free Radic Biol Med* 105:93–101. <https://doi.org/10.1016/j.freeradbiomed.2016.09.022>.
  156. Warburg O, Wind F, Negelein E. 1927. The metabolism of tumors in the body. *J Gen Physiol* 8:519–530. <https://doi.org/10.1085/jgp.8.6.519>.
  157. Barrow PA, Berchieri A, Freitas Neto OC, Lovell M. 2015. The contribution of aerobic and anaerobic respiration to intestinal colonization and virulence for *Salmonella* Typhimurium in the chicken. *Avian Pathol* 44:401–407. <https://doi.org/10.1080/03079457.2015.1062841>.
  158. Ley RE, Hamady M, Lozupone C, Turnbaugh PJ, Ramey RR, Birchler JS, Schlegel ML, Tucker TA, Schrenzel MD, Knight R, Gordon JL. 2008. Evolution of mammals and their gut microbes. *Science* 320:1647–1651. <https://doi.org/10.1126/science.1155725>.
  159. Orcutt RP, Gianni FJ, Judge RJ. 1987. Development of an altered Schaedler flora for NCI gnotobiotic rodents. *Microecol Ther* 17:59–59.
  160. Brand MW, Wannemuehler MJ, Phillips GJ, Proctor A, Overstreet AM, Jergens AE, Orcutt RP, Fox JG. 2015. The altered Schaedler flora: continued applications of a defined murine microbial community. *ILAR J* 56:169–178. <https://doi.org/10.1093/ilar/ilv012>.
  161. Lima-Filho JV, Vieira LQ, Arantes RM, Nicolli JR. 2004. Effect of the *Escherichia coli* EMO strain on experimental infection by *Salmonella enterica* serovar Typhimurium in gnotobiotic mice. *Braz J Med Biol Res* 37:1005–1013. <https://doi.org/10.1590/S0100-879X2004000700009>.
  162. Splichalova A, Trebichavsky I, Rada V, Vlkova E, Sonnenborn U, Splichal I. 2011. Interference of *Bifidobacterium choerinum* or *Escherichia coli* Nissle 1917 with *Salmonella* Typhimurium in gnotobiotic piglets correlates with cytokine patterns in blood and intestine. *Clin Exp Immunol* 163:242–249. <https://doi.org/10.1111/j.1365-2249.2010.04283.x>.
  163. Wotzka SY, Kreuzer M, Maier L, Arnoldini M, Nguyen BD, Brachmann AO, Berthold DL, Zund M, Hausmann A, Bakkeren E, Hoces D, Gul E, Beutler M, Dolowschiak T, Zimmermann M, Fuhrer T, Moor K, Sauer U, Typas A, Piel J, Diard M, Macpherson AJ, Stecher B, Sunagawa S, Slack E, Hardt WD. 2019. *Escherichia coli* limits *Salmonella* Typhimurium infections after diet shifts and fat-mediated microbiota perturbation in mice. *Nat Microbiol* 4:2164–2174. <https://doi.org/10.1038/s41564-019-0568-5>.
  164. Brugiroux S, Beutler M, Pfann C, Garzetti D, Ruscheweyh HJ, Ring D, Diehl M, Herp S, Lotscher Y, Hussain S, Bunk B, Pukall R, Huson DH, Munch PC, McHardy AC, McCoy KD, Macpherson AJ, Loy A, Clavel T, Berry D, Stecher

- B. 2016. Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella enterica* serovar Typhimurium. *Nat Microbiol* 2:16215. <https://doi.org/10.1038/nmicrobiol.2016.215>.
165. Ng KM, Ferreyra JA, Higginbottom SK, Lynch JB, Kashyap PC, Gopinath S, Naidu N, Choudhury B, Weimer BC, Monack DM, Sonnenburg JL. 2013. Microbiota-liberated host sugars facilitate post-antibiotic expansion of enteric pathogens. *Nature* 502:96–99. <https://doi.org/10.1038/nature12503>.
166. Stecher B, Barthel M, Schlumberger MC, Haberli L, Rabsch W, Kremer M, Hardt WD. 2008. Motility allows *S. Typhimurium* to benefit from the mucosal defence. *Cell Microbiol* 10:1166–1180. <https://doi.org/10.1111/j.1462-5822.2008.01118.x>.
167. Faber F, Tran L, Byndloss MX, Lopez CA, Velazquez EM, Kerrinnes T, Nuccio SP, Wangdi T, Fiehn O, Tsois RM, Baumler AJ. 2016. Host-mediated sugar oxidation promotes post-antibiotic pathogen expansion. *Nature* 534:697–699. <https://doi.org/10.1038/nature18597>.
168. Spees AM, Wangdi T, Lopez CA, Kingsbury DD, Xavier MN, Winter SE, Tsois RM, Baumler AJ. 2013. Streptomycin-induced inflammation enhances *Escherichia coli* gut colonization through nitrate respiration. *mBio* 4:e00430-13. <https://doi.org/10.1128/mBio.00430-13>.
169. Oliveira RA, Ng KM, Correia MB, Cabral V, Shi H, Sonnenburg JL, Huang KC, Xavier KB. 2020. *Klebsiella michiganensis* transmission enhances resistance to *Enterobacteriaceae* gut invasion by nutrition competition. *Nat Microbiol* 5:630–641. <https://doi.org/10.1038/s41564-019-0658-4>.
170. Daikos GL, Vryonis E, Psychogiou M, Tzouveleki LS, Liatis S, Petrikos P, Kosmidis C, Tassios PT, Bamias G, Skoutelis A. 2010. Risk factors for bloodstream infection with *Klebsiella pneumoniae* producing VIM-1 metallo-beta-lactamase. *J Antimicrob Chemother* 65:784–788. <https://doi.org/10.1093/jac/dkq005>.
171. Shanthi M, Sekar U. 2010. Extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. *J Assoc Physicians India* 58(Suppl):41–44.
172. Razzazi K, Derde LP, Verachten M, Legrand P, Lesprit P, Brun-Buisson C. 2012. Clinical impact and risk factors for colonization with extended-spectrum beta-lactamase-producing bacteria in the intensive care unit. *Intensive Care Med* 38:1769–1778. <https://doi.org/10.1007/s00134-012-2675-0>.
173. Lowe CF, Kus JV, Salt N, Callery S, Louie L, Khan MA, Vearncombe M, Simor AE. 2013. Nosocomial transmission of New Delhi metallo-beta-lactamase-1-producing *Klebsiella pneumoniae* in Toronto, Canada. *Infect Control Hosp Epidemiol* 34:49–55. <https://doi.org/10.1086/668778>.
174. Zhao SY, Zhang J, Zhang YL, Wang YC, Xiao SZ, Gu FF, Guo XK, Ni YX, Han LZ. 2016. Epidemiology and risk factors for faecal extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E) carriage derived from residents of seven nursing homes in western Shanghai, China. *Epidemiol Infect* 144:695–702. <https://doi.org/10.1017/S0950268815001879>.
175. Gorrie CL, Mirceta M, Wick RR, Edwards DJ, Thomson NR, Strugnell RA, Pratt NF, Garlick JS, Watson KM, Pilcher DV, McGloughlin SA, Spelman DW, Jenney AWJ, Holt KE. 2017. Gastrointestinal carriage is a major reservoir of *Klebsiella pneumoniae* infection in intensive care patients. *Clin Infect Dis* 65:208–215. <https://doi.org/10.1093/cid/cix270>.
176. Tamburini FB, Andermann TM, Tkachenko E, Senchyna F, Banaei N, Bhatt AS. 2018. Precision identification of diverse bloodstream pathogens in the gut microbiome. *Nat Med* 24:1809–1814. <https://doi.org/10.1038/s41591-018-0202-8>.
177. Doi Y, Paterson DL. 2015. Carbapenemase-producing *Enterobacteriaceae*. *Semin Respir Crit Care Med* 36:74–84. <https://doi.org/10.1055/s-0035-1544208>.
178. Tangden T, Giske CG. 2015. Global dissemination of extensively drug-resistant carbapenemase-producing *Enterobacteriaceae*: clinical perspectives on detection, treatment, and infection control. *J Intern Med* 277:501–512. <https://doi.org/10.1111/joim.12342>.
179. Centers for Disease Control and Prevention. 2014. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, Atlanta, GA. <http://www.cdc.gov/drugresistance/threat-report-2013/index.html>.
180. Galán JE, Curtiss IIR. 1989. Cloning and molecular characterization of genes whose products allow *Salmonella* Typhimurium to penetrate tissue culture cells. *Proc Natl Acad Sci U S A* 86:6383–6387. <https://doi.org/10.1073/pnas.86.16.6383>.
181. Hensel M, Shea JE, Gleeson C, Jones MD, Dalton E, Holden DW. 1995. Simultaneous identification of bacterial virulence genes by negative selection. *Science* 269:400–403. <https://doi.org/10.1126/science.7618105>.
182. Tsois RM, Adams LG, Ficht TA, Baumler AJ. 1999. Contribution of *Salmonella* Typhimurium virulence factors to diarrheal disease in calves. *Infect Immun* 67:4879–4885. <https://doi.org/10.1128/IAI.67.9.4879-4885.1999>.
183. Costello EK, Stagaman K, Dethlefsen L, Bohannan BJ, Relman DA. 2012. The application of ecological theory toward an understanding of the human microbiome. *Science* 336:1255–1262. <https://doi.org/10.1126/science.1224203>.
184. Srikanth CV, Mercado-Lubo R, Hallstrom K, McCormick BA. 2011. *Salmonella* effector proteins and host-cell responses. *Cell Mol Life Sci* 68:3687–3697. <https://doi.org/10.1007/s00018-011-0841-0>.
185. Raffatellu M, Wilson RP, Chessa D, Andrews-Polymenis H, Tran QT, Lawhorn S, Khare S, Adams LG, Baumler AJ. 2005. SipA, SopA, SopB, SopD, and SopE2 contribute to *Salmonella enterica* serotype Typhimurium invasion of epithelial cells. *Infect Immun* 73:146–154. <https://doi.org/10.1128/IAI.73.1.146-154.2005>.
186. Grant AJ, Morgan FJ, McKinley TJ, Foster GL, Maskell DJ, Mastroeni P. 2012. Attenuated *Salmonella* Typhimurium lacking the pathogenicity island-2 type 3 secretion system grow to high bacterial numbers inside phagocytes in mice. *PLoS Pathog* 8:e1003070. <https://doi.org/10.1371/journal.ppat.1003070>.
187. LaRock DL, Chaudhary A, Miller SI. 2015. Salmonellae interactions with host processes. *Nat Rev Microbiol* 13:191–205. <https://doi.org/10.1038/nrmicro3420>.
188. Tukul C, Raffatellu M, Humphries AD, Wilson RP, Andrews-Polymenis HL, Gull T, Figueiredo JF, Wong MH, Michelsen KS, Akcelik M, Adams LG, Baumler AJ. 2005. CsgA is a pathogen-associated molecular pattern of *Salmonella enterica* serotype Typhimurium that is recognized by Toll-like receptor 2. *Mol Microbiol* 58:289–304. <https://doi.org/10.1111/j.1365-2958.2005.04825.x>.
189. Keestra AM, Godinez I, Xavier MN, Winter MG, Winter SE, Tsois RM, Baumler AJ. 2011. Early MyD88-dependent induction of interleukin-17A expression during *Salmonella* colitis. *Infect Immun* 79:3131–3140. <https://doi.org/10.1128/IAI.00018-11>.
190. Keestra AM, Winter MG, Klein-Douwel D, Xavier MN, Winter SE, Kim A, Tsois RM, Baumler AJ. 2011. A *Salmonella* virulence factor activates the NOD1/NOD2 signaling pathway. *mBio* 2:e00266-11. <https://doi.org/10.1128/mBio.00266-11>.
191. Keestra AM, Winter MG, Auburger JJ, Frassle SP, Xavier MN, Winter SE, Kim A, Poon V, Ravesloot MM, Waldenmaier JF, Tsois RM, Eigenheer RA, Baumler AJ. 2013. Manipulation of small Rho GTPases is a pathogen-induced process detected by NOD1. *Nature* 496:233–237. <https://doi.org/10.1038/nature12025>.
192. Sellin ME, Muller AA, Felmy B, Dolowschiak T, Diard M, Tardivel A, Maslowski KM, Hardt WD. 2014. Epithelium-intrinsic NAIP/NLRC4 inflammasome drives infected enterocyte expulsion to restrict *Salmonella* replication in the intestinal mucosa. *Cell Host Microbe* 16:237–248. <https://doi.org/10.1016/j.chom.2014.07.001>.
193. Knodler LA, Crowley SM, Sham HP, Yang H, Wrangle M, Ma C, Ernst RK, Steele-Mortimer O, Celli J, Vallance BA. 2014. Noncanonical inflammasome activation of caspase-4/caspase-11 mediates epithelial defenses against enteric bacterial pathogens. *Cell Host Microbe* 16:249–256. <https://doi.org/10.1016/j.chom.2014.07.002>.
194. Zhang S, Santos RL, Tsois RM, Stender S, Hardt WD, Baumler AJ, Adams LG. 2002. The *Salmonella enterica* serotype Typhimurium effector proteins SipA, SopA, SopB, SopD, and SopE2 act in concert to induce diarrhea in calves. *Infect Immun* 70:3843–3855. <https://doi.org/10.1128/iai.70.7.3843-3855.2002>.
195. Matsuda S, Haneda T, Saito H, Miki T, Okada N. 2019. *Salmonella enterica* effectors SifA, SpvB, SseF, SseJ, and SteA contribute to type III secretion system 1-independent inflammation in a streptomycin-pretreated mouse model of colitis. *Infect Immun* 87:e00872-18. <https://doi.org/10.1128/IAI.00872-18>.
196. Harris JC, Dupont HL, Hornick RB. 1972. Fecal leukocytes in diarrheal illness. *Ann Intern Med* 76:697–703. <https://doi.org/10.7326/0003-4819-76-5-697>.
197. Sekirov I, Gill N, Jogova M, Tam N, Robertson M, de Llanos R, Li Y, Finlay BB. 2010. *Salmonella* SPI-1-mediated neutrophil recruitment during enteric colitis is associated with reduction and alteration in intestinal microbiota. *Gut Microbes* 1:30–41. <https://doi.org/10.4161/gmic.1.1.10950>.
198. Gill N, Ferreira RB, Antunes LC, Willing BP, Sekirov I, Al-Zahrani F, Hartmann M, Finlay BB. 2012. Neutrophil elastase alters the murine gut microbiota resulting in enhanced *Salmonella* colonization. *PLoS One* 7:e49646. <https://doi.org/10.1371/journal.pone.0049646>.
199. Lawley TD, Bouley DM, Hoy YE, Gerke C, Relman DA, Monack DM. 2008.



- Host transmission of *Salmonella enterica* serovar Typhimurium is controlled by virulence factors and indigenous intestinal microbiota. *Infect Immun* 76:403–416. <https://doi.org/10.1128/IAI.01189-07>.
200. Crawford RW, Keestra AM, Winter SE, Xavier MN, Tsois RM, Tolstikov V, Baumler AJ. 2012. Very long O-antigen chains enhance fitness during *Salmonella*-induced colitis by increasing bile resistance. *PLoS Pathog* 8:e1002918. <https://doi.org/10.1371/journal.ppat.1002918>.
201. Sana TG, Flaughnatti N, Lugo KA, Lam LH, Jacobson A, Baylot V, Durand E, Journet L, Cascales E, Monack DM. 2016. *Salmonella* Typhimurium utilizes a T6SS-mediated antibacterial weapon to establish in the host gut. *Proc Natl Acad Sci U S A* 113:E5044–E5051. <https://doi.org/10.1073/pnas.1608858113>.
202. Costa LF, Mol JP, Silva AP, Macedo AA, Silva TM, Alves GE, Winter S, Winter MG, Velazquez EM, Byndloss MX, Baumler AJ, Tsois RM, Paixao TA, Santos RL. 2016. Iron acquisition pathways and colonization of the inflamed intestine by *Salmonella enterica* serovar Typhimurium. *Int J Med Microbiol* 306:604–610. <https://doi.org/10.1016/j.ijmm.2016.10.004>.
203. Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. 2002. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 10:1033–1043. [https://doi.org/10.1016/s1097-2765\(02\)00708-6](https://doi.org/10.1016/s1097-2765(02)00708-6).
204. Flo TH, Smith KD, Sato S, Rodriguez DJ, Holmes MA, Strong RK, Akira S, Aderem A. 2004. Lipocalin 2 mediates an innate immune response to bacterial infection by sequestering iron. *Nature* 432:917–921. <https://doi.org/10.1038/nature03104>.
205. Raffatellu M, George MD, Akiyama Y, Hornsby MJ, Nuccio SP, Paixao TA, Butler BP, Chu H, Santos RL, Berger T, Mak TW, Tsois RM, Bevins CL, Solnick JV, Dandekar S, Baumler AJ. 2009. Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype Typhimurium for growth and survival in the inflamed intestine. *Cell Host Microbe* 5:476–486. <https://doi.org/10.1016/j.chom.2009.03.011>.
206. Chassaing B, Srinivasan G, Delgado MA, Young AN, Gewirtz AT, Vijay-Kumar M. 2012. Fecal lipocalin 2, a sensitive and broadly dynamic noninvasive biomarker for intestinal inflammation. *PLoS One* 7:e44328. <https://doi.org/10.1371/journal.pone.0044328>.
207. Hantke K, Nicholson G, Rabsch W, Winkelmann G. 2003. Salmochelins, siderophores of *Salmonella enterica* and uropathogenic *Escherichia coli* strains, are recognized by the outer membrane receptor IronN. *Proc Natl Acad Sci U S A* 100:3677–3682. <https://doi.org/10.1073/pnas.0737682100>.
208. Fischbach MA, Lin H, Zhou L, Yu Y, Abergel RJ, Liu DR, Raymond KN, Wanner BL, Strong RK, Walsh CT, Aderem A, Smith KD. 2006. The pathogen-associated *iroA* gene cluster mediates bacterial evasion of lipocalin 2. *Proc Natl Acad Sci U S A* 103:16502–16507. <https://doi.org/10.1073/pnas.0604636103>.
209. Behnsen J, Jellbauer S, Wong CP, Edwards RA, George MD, Ouyang W, Raffatellu M. 2014. The cytokine IL-22 promotes pathogen colonization by suppressing related commensal bacteria. *Immunity* 40:262–273. <https://doi.org/10.1016/j.immuni.2014.01.003>.
210. Patzer SI, Baquero MR, Bravo D, Moreno F, Hantke K. 2003. The colicin G, H and X determinants encode microcins M and H47, which might utilize the catecholate siderophore receptors FepA, Cir, Fiu, and IronN. *Microbiology (Reading)* 149:2557–2570. <https://doi.org/10.1099/mic.0.26396-0>.
211. Sassone-Corsi M, Nuccio SP, Liu H, Hernandez D, Vu CT, Takahashi AA, Edwards RA, Raffatellu M. 2016. Microcins mediate competition among *Enterobacteriaceae* in the inflamed gut. *Nature* 540:280–283. <https://doi.org/10.1038/nature20557>.
212. Nedialkova LP, Denzler R, Koeppl MB, Diehl M, Ring D, Wille T, Gerlach RG, Stecher B. 2014. Inflammation fuels colicin Ib-dependent competition of *Salmonella* serovar Typhimurium and *Escherichia coli* in enterobacterial blooms. *PLoS Pathog* 10:e1003844. <https://doi.org/10.1371/journal.ppat.1003844>.
213. Nuccio SP, Baumler AJ. 2014. Comparative analysis of *Salmonella* genomes identifies a metabolic network for escalating growth in the inflamed gut. *mBio* 5:e00929-14. <https://doi.org/10.1128/mBio.00929-14>.
214. Seif Y, Kavvas E, Lachance JC, Yurkovich JT, Nuccio SP, Fang X, Catoiu E, Raffatellu M, Palsson BO, Monk JM. 2018. Genome-scale metabolic reconstructions of multiple *Salmonella* strains reveal serovar-specific metabolic traits. *Nat Commun* 9:3771. <https://doi.org/10.1038/s41467-018-06112-5>.
215. Wheeler NE, Gardner PP, Barquist L. 2018. Machine learning identifies signatures of host adaptation in the bacterial pathogen *Salmonella enterica*. *PLoS Genet* 14:e1007333. <https://doi.org/10.1371/journal.pgen.1007333>.
216. Raffatellu M, Wilson RP, Winter SE, Baumler AJ. 2008. Clinical pathogenesis of typhoid fever. *J Infect Dev Ctries* 2:260–266. <https://doi.org/10.3855/jidc.219>.
217. Rakov AV, Mastriani E, Liu SL, Schifferli DM. 2019. Association of *Salmonella* virulence factor alleles with intestinal and invasive serovars. *BMC Genomics* 20:429. <https://doi.org/10.1186/s12864-019-5809-8>.
218. Parkhill J, Dougan G, James KD, Thomson NR, Pickard D, Wain J, Churcher C, Mungall KL, Bentley SD, Holden MT, Sebahia M, Baker S, Basham D, Brooks K, Chillingworth T, Connor P, Cronin A, Davis P, Davies RM, Dowd L, White N, Farrar J, Feltwell T, Hamlin N, Haque A, Hien TT, Holroyd S, Jagels K, Krogh A, Larsen TS, Leather S, Moule S, O'Gaora P, Parry C, Quail M, Rutherford K, Simmonds M, Skelton J, Stevens K, Whitehead S, Barrell BG. 2001. Complete genome sequence of a multiple drug-resistant *Salmonella enterica* serovar Typhi CT18. *Nature* 413:848–852. <https://doi.org/10.1038/351101607>.
219. Deng W, Liou SR, Plunkett G, Ill, Mayhew GF, Rose DJ, Burland V, Kodoyianni V, Schwartz DC, Blattner FR. 2003. Comparative genomics of *Salmonella enterica* serovar Typhi strains Ty2 and CT18. *J Bacteriol* 185:2330–2337. <https://doi.org/10.1128/jb.185.7.2330-2337.2003>.
220. Thomson NR, Clayton DJ, Windhorst D, Vernikos G, Davidson S, Churcher C, Quail MA, Stevens M, Jones MA, Watson M, Barron A, Layton A, Pickard D, Kingsley RA, Bignell A, Clark L, Harris B, Ormond D, Abdellah Z, Brooks K, Cherevach I, Chillingworth T, Woodward J, Norbertczak H, Lord A, Arrowsmith C, Jagels K, Moule S, Mungall K, Sanders M, Whitehead S, Chabalgoity JA, Maskell D, Humphrey T, Roberts M, Barrow PA, Dougan G, Parkhill J. 2008. Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. *Genome Res* 18:1624–1637. <https://doi.org/10.1101/gr.077404.108>.
221. Holt KE, Thomson NR, Wain J, Langridge GC, Hasan R, Bhutta ZA, Quail MA, Norbertczak H, Walker D, Simmonds M, White B, Bason N, Mungall K, Dougan G, Parkhill J. 2009. Pseudogene accumulation in the evolutionary histories of *Salmonella enterica* serovars Paratyphi A and Typhi. *BMC Genomics* 10:36. <https://doi.org/10.1186/1471-2164-10-36>.
222. Liu WQ, Feng Y, Wang Y, Zou QH, Chen F, Guo JT, Peng YH, Jin Y, Li YG, Hu SN, Johnston RN, Liu GR, Liu SL. 2009. *Salmonella* Paratyphi C: genetic divergence from *Salmonella choleraesuis* and pathogenic convergence with *Salmonella* Typhi. *PLoS One* 4:e4510. <https://doi.org/10.1371/journal.pone.0004510>.
223. Langridge GC, Fookes M, Connor TR, Feltwell T, Feasey N, Parsons BN, Seth-Smith HM, Barquist L, Stedman A, Humphrey T, Wigley P, Peters SE, Maskell DJ, Corander J, Chabalgoity JA, Barrow P, Parkhill J, Dougan G, Thomson NR. 2015. Patterns of genome evolution that have accompanied host adaptation in *Salmonella*. *Proc Natl Acad Sci U S A* 112:863–868. <https://doi.org/10.1073/pnas.1416707112>.
224. Matthews TD, Schmieder R, Silva GG, Busch J, Cassman N, Dutilh BE, Green D, Matlock B, Heffernan B, Olsen GJ, Farris Hanna L, Schifferli DM, Maloy S, Dinsdale EA, Edwards RA. 2015. Genomic comparison of the closely-related *Salmonella enterica* serovars Enteritidis, Dublin, and Gallinarum. *PLoS One* 10:e0126883. <https://doi.org/10.1371/journal.pone.0126883>.
225. Key FM, Posth C, Esquivel-Gomez LR, Hubler R, Spyrou MA, Neumann GU, Furtwangler A, Sabin S, Burri M, Wissgott A, Lankapalli AK, Vagene AJ, Meyer M, Nagel S, Tukhbatova R, Khokhlov A, Chizhevsky A, Hansen S, Belinsky AB, Kalmykov A, Kantorovich AR, Maslov VE, Stockhammer PW, Vai S, Zavattaro M, Riga A, Caramelli D, Skeates R, Beckett J, Gradoli MG, Steuri N, Hafner A, Ramstein M, Siebke I, Losch S, Erdal YS, Alikhan NF, Zhou Z, Achtman M, Bos K, Reinhold S, Haak W, Kuhnert D, Herbig A, Krause J. 2020. Emergence of human-adapted *Salmonella enterica* is linked to the neolithization process. *Nat Ecol Evol* 4:324–333. <https://doi.org/10.1038/s41559-020-1106-9>.
226. Hu Y, Wang Z, Qiang B, Xu Y, Chen X, Li Q, Jiao X. 2019. Loss and gain in the evolution of the *Salmonella enterica* serovar Gallinarum biovar Pullorum genome. *mSphere* 4:e00627-18. <https://doi.org/10.1128/mSphere.00627-18>.
227. Maier L, Vyas R, Cordova CD, Lindsay H, Schmidt TS, Brugiroux S, Periaswamy B, Bauer R, Sturm A, Schreiber F, von Mering C, Robinson MD, Stecher B, Hardt WD. 2013. Microbiota-derived hydrogen fuels *Salmonella* Typhimurium invasion of the gut ecosystem. *Cell Host Microbe* 14:641–651. <https://doi.org/10.1016/j.chom.2013.11.002>.
228. Rivera-Chavez F, Winter SE, Lopez CA, Xavier MN, Winter MG, Nuccio SP, Russell JM, Laughlin RC, Lawhon SD, Stenzenbach T, Bevins CL, Tsois RM, Harshey R, Adams LG, Baumler AJ. 2013. *Salmonella* uses energy taxis to benefit from intestinal inflammation. *PLoS Pathog* 9:e1003267. <https://doi.org/10.1371/journal.ppat.1003267>.
229. Faber F, Thiennimitr P, Spiga L, Byndloss MX, Litvak Y, Lawhon S,

- Andrews-Polymenis HL, Winter SE, Baumler AJ. 2017. Respiration of microbiota-derived 1,2-propanediol drives *Salmonella* expansion during colitis. *PLoS Pathog* 13:e1006129. <https://doi.org/10.1371/journal.ppat.1006129>.
230. Thiennimitr P, Winter SE, Winter MG, Xavier MN, Tolstikov V, Huseby DL, Sterzenbach T, Tsois RM, Roth JR, Baumler AJ. 2011. Intestinal inflammation allows *Salmonella* to use ethanolamine to compete with the microbiota. *Proc Natl Acad Sci U S A* 108:17480–17485. <https://doi.org/10.1073/pnas.1107857108>.
231. Spiga L, Winter MG, Furtado de Carvalho T, Zhu W, Hughes ER, Gillis CC, Behrendt CL, Kim J, Chessa D, Andrews-Polymenis HL, Beiting DP, Santos RL, Hooper LV, Winter SE. 2017. An oxidative central metabolism enables *Salmonella* to utilize microbiota-derived succinate. *Cell Host Microbe* 22:291–301 e6. <https://doi.org/10.1016/j.chom.2017.07.018>.
232. Campbell JW, Morgan-Kiss RM, Cronan JE, Jr. 2003. A new *Escherichia coli* metabolic competency: growth on fatty acids by a novel anaerobic beta-oxidation pathway. *Mol Microbiol* 47:793–805. <https://doi.org/10.1046/j.1365-2958.2003.03341.x>.
233. Bronner DN, Faber F, Olsan EE, Byndloss MX, Sayed NA, Xu G, Yoo W, Kim D, Ryu S, Lebrilla CB, Baumler AJ. 2018. Genetic ablation of butyrate utilization attenuates gastrointestinal *Salmonella* disease. *Cell Host Microbe* 23:266–273. <https://doi.org/10.1016/j.chom.2018.01.004>.
234. Schoneich C. 2005. Methionine oxidation by reactive oxygen species: reaction mechanisms and relevance to Alzheimer's disease. *Biochim Biophys Acta* 1703:111–119. <https://doi.org/10.1016/j.bbapap.2004.09.009>.
235. Balagam B, Richardson DE. 2008. The mechanism of carbon dioxide catalysis in the hydrogen peroxide N-oxidation of amines. *Inorg Chem* 47:1173–1178. <https://doi.org/10.1021/ic701402h>.
236. Heinzinger NK, Fujimoto SY, Clark MA, Moreno MS, Barrett EL. 1995. Sequence analysis of the *phs* operon in *Salmonella* Typhimurium and the contribution of thiosulfate reduction to anaerobic energy metabolism. *J Bacteriol* 177:2813–2820. <https://doi.org/10.1128/jb.177.10.2813-2820.1995>.
237. Rizzatti G, Lopetuso LR, Gibiino G, Binda C, Gasbarrini A. 2017. Proteobacteria: a common factor in human diseases. *Biomed Res Int* 2017:9351507. <https://doi.org/10.1155/2017/9351507>.
238. Zhu W, Miyata N, Winter MG, Arenales A, Hughes ER, Spiga L, Kim J, Sifuentes-Dominguez L, Starokadomskyy P, Gopal P, Byndloss MX, Santos RL, Burstein E, Winter SE. 2019. Editing of the gut microbiota reduces carcinogenesis in mouse models of colitis-associated colorectal cancer. *J Exp Med* 216:2378–2393. <https://doi.org/10.1084/jem.20181939>.
239. Bakken JS, Borody T, Brandt LJ, Brill JV, Demarco DC, Franzos MA, Kelly C, Khoruts A, Louie T, Martinelli LP, Moore TA, Russell G, Surawicz C, Fecal Microbiota Transplantation W, Fecal Microbiota Transplantation Workgroup. 2011. Treating *Clostridium difficile* infection with fecal microbiota transplantation. *Clin Gastroenterol Hepatol* 9:1044–1049. <https://doi.org/10.1016/j.cgh.2011.08.014>.
240. Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gouberne A, No D, Liu H, Kinnebrew M, Viale A, Littmann E, van den Brink MR, Jenq RR, Taur Y, Sander C, Cross JR, Toussaint NC, Xavier JB, Pamer EG. 2015. Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. *Nature* 517:205–208. <https://doi.org/10.1038/nature13828>.
241. Khan KJ, Ullman TA, Ford AC, Abreu MT, Abadir A, Abadir A, Marshall JK, Talley NJ, Moayyedi P. 2011. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 106:661–673. <https://doi.org/10.1038/ajg.2011.72>.
242. Hildebrand H, Malmborg P, Askling J, Ekbo M, Montgomery SM. 2008. Early-life exposures associated with antibiotic use and risk of subsequent Crohn's disease. *Scand J Gastroenterol* 43:961–966. <https://doi.org/10.1080/00365520801971736>.
243. Hviid A, Svanstrom H, Frisch M. 2011. Antibiotic use and inflammatory bowel diseases in childhood. *Gut* 60:49–54. <https://doi.org/10.1136/gut.2010.219683>.
244. Zou Y, Wu L, Xu W, Zhou X, Ye K, Xiong H, Song C, Xie Y. 2020. Correlation between antibiotic use in childhood and subsequent inflammatory bowel disease: a systematic review and meta-analysis. *Scand J Gastroenterol* 55:301–311. <https://doi.org/10.1080/00365521.2020.1737882>.
245. Xu J, Chen N, Wu Z, Song Y, Zhang Y, Wu N, Zhang F, Ren X, Liu Y. 2018. 5-Aminosalicylic acid alters the gut bacterial microbiota in patients with ulcerative colitis. *Front Microbiol* 9:1274. <https://doi.org/10.3389/fmicb.2018.01274>.
246. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunker MK, Bultman SJ. 2011. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metab* 13:517–526. <https://doi.org/10.1016/j.cmet.2011.02.018>.
247. Donohoe DR, Wali A, Brylawski BP, Bultman SJ. 2012. Microbial regulation of glucose metabolism and cell-cycle progression in mammalian colonocytes. *PLoS One* 7:e46589. <https://doi.org/10.1371/journal.pone.0046589>.
248. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A, Weir TL, Ehrentraut SF, Pickel C, Kuhn KA, Lanis JM, Nguyen V, Taylor CT, Colgan SP. 2015. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial HIF augments tissue barrier function. *Cell Host Microbe* 17:662–671. <https://doi.org/10.1016/j.chom.2015.03.005>.
249. Pereira IA, Ramos AR, Grein F, Marques MC, da Silva SM, Venceslau SS. 2011. A comparative genomic analysis of energy metabolism in sulfate reducing bacteria and archaea. *Front Microbiol* 2:69. <https://doi.org/10.3389/fmicb.2011.00069>.
250. Furne J, Springfield J, Koenig T, DeMaster E, Levitt MD. 2001. Oxidation of hydrogen sulfide and methanethiol to thiosulfate by rat tissues: a specialized function of the colonic mucosa. *Biochem Pharmacol* 62:255–259. [https://doi.org/10.1016/s0006-2952\(01\)00657-8](https://doi.org/10.1016/s0006-2952(01)00657-8).
251. Levitt MD, Furne J, Springfield J, Suarez F, DeMaster E. 1999. Detoxification of hydrogen sulfide and methanethiol in the cecal mucosa. *J Clin Invest* 104:1107–1114. <https://doi.org/10.1172/JCI7712>.
252. Palmer RM, Ashton DS, Moncada S. 1988. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 333:664–666. <https://doi.org/10.1038/333664a0>.
253. De Groote MA, Granger D, Xu Y, Campbell G, Prince R, Fang FC. 1995. Genetic and redox determinants of nitric oxide cytotoxicity in a *Salmonella* Typhimurium model. *Proc Natl Acad Sci U S A* 92:6399–6403. <https://doi.org/10.1073/pnas.92.14.6399>.
254. Szabo C, Ischiropoulos H, Radi R. 2007. Peroxynitrite: biochemistry, pathophysiology, and development of therapeutics. *Nat Rev Drug Discov* 6:662–680. <https://doi.org/10.1038/nrd2222>.