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How dysbiosis might contribute to pathogen evolution

Review Article**Author(s):**

Stecher, Bärbel; Maier, Lisa; [Hardt, Wolf-Dietrich](#) 

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“Blooming” in the gut: how dysbiosis may contribute to pathogen evolution

5 **Autors:** Bärbel Stecher^{1,2}, Lisa Maier³ and Wolf-Dietrich Hardt^{3*}

6 ¹Max von Pettenkofer Institut, LMU München, Germany; ²German Center for Infection Research,
7 LMU München; ³Institute of Microbiology, ETH Zürich, Switzerland.

8 * Correspondence to W-D H

9 **Preface:**

10 Hundreds of bacterial species make up the mammalian intestinal microbiota. Upon perturbations by
11 antibiotics, diet, immune deficiency or infection, this ecosystem can shift to a state of dysbiosis. This
12 may involve overgrowth (‘blooming’) of otherwise underrepresented or potentially harmful bacteria
13 (for example, pathobionts). Here we present evidence suggesting that dysbiosis may fuel horizontal
14 gene transfer between members of this ecosystem, facilitating the transfer of virulence and antibiotic
15 resistance genes, thereby promoting pathogen evolution.

16 **Introduction**

17 In 1996, Rodney T. Berg coined the following calculation: “In summary, there are ... 10^{14} total
18 gastrointestinal tract (GI) bacteria If we assume that one mutation in every 10^8 bacterial divisions
19 is a viable mutation, 10^{14} total bacteria in the GI tract theoretically will produce 10^6 newly mutated
20 viable bacteria at every division cycle. It is estimated that the bacteria in the GI tract divide every 20
21 minutes. This generation of large numbers of newly mutated bacteria at every division cycle allows
22 the indigenous GI microflora to adapt rapidly to GI environmental changes”¹.

23 Given the fact that we are looking back on millions of years of microbiota-host co-evolution,
24 consistently starting at birth with a first encounter of the microbiota, the combination of high bacterial
25 numbers and their relatively short generation times implies that any contemporary microbiota can be
26 regarded as the “snapshot” of a vast and continuing evolutionary process. Seminal contributions by
27 large-scale sequencing consortia in the past years have offered unprecedented insights into the human
28 microbiota’s assembly, individuality and stability over time ²⁻⁶. Functional changes in the microbiota
29 can derive from variations in the microbial transcriptome, proteome or metabolome. Altered
30 microbiota functionality can also be introduced by diversification of the collective microbial gene
31 pool (the microbiome), which can occur at three different levels: by intrusion or disappearance of
32 individual members (for example, invading bacterial strains during maturation of an infant gut
33 microbiota) ⁷; by shifts in relative bacterial abundances such as those caused by dietary changes,

1 immune deficiency, antibiotic use or infections^{8, 9}, potentially leading to dysbiosis (**Box 1**); and
2 finally by mutation or horizontal gene transfer (HGT). These alterations can significantly impact the
3 overall functionality of the microbiota, by enhancing the individual fitness of certain keystone
4 pathogens or keystone stabilizers (**Figure 1**).

5 HGT in particular enables bacterial evolution in quantum leaps rather than by step-wise adaptation
6 through mutations, drive shifts in community composition and can potentially shift the system into
7 dysbiosis. In the mammalian gut, HGT is thought to occur at a higher frequency than in other
8 microbial ecosystems. This has essentially been attributed to its enormous local bacterial density^{10, 11}.
9 To date, the actual frequency of genetic exchange, its hotspots and limitations in terms of species
10 boundaries and their contribution to overall ecosystem functionality can only be estimated from
11 anecdotal evidence. Recent studies have established that environmental changes and microbiota
12 perturbations can have profound and long-lasting effects on microbiota community structure and
13 foster ‘blooms’ of otherwise low-abundant bacteria (**Table 1**). In particular, infections with enteric
14 pathogens can give rise to enterobacterial blooms¹²⁻¹⁴, which can boost HGT between pathogenic and
15 commensal bacterial species¹⁵. It seems reasonable to assume that bloom-driven HGT may promote
16 bacterial evolution in the aftermath of such perturbations.

17 In this Opinion article, we argue that intestinal dysbiosis may act as a driver of HGT in the gut
18 ecosystem, promoting pathogen evolution and the spread of antibiotic resistances.

19 **HGT in the gut ecosystem**

20 HGT-mediated acquisition of ‘ready-made’ genes, entire metabolic pathways, fitness and virulence
21 factors as well as antibiotic resistance genes enables a swift adaptation of microbial communities to
22 changing environmental conditions. Below we discuss mechanisms of HGT and why this process
23 might be favoured in the conditions present in the intestine. *Mechanisms of HGT*. HGT is most
24 efficient among closely related species, but can also occur between distantly related bacteria:
25 *Enterobacteriaceae* can undergo HGT with Gram-positive species from the *Firmicutes* division¹⁶⁻¹⁸.
26 Even inter-kingdom HGT has been described¹⁹. In general, HGT in bacteria can take place by three
27 different mechanisms: transformation, transduction via bacteriophages and conjugation-mediated
28 plasmid-exchange (**Figure 2A**).

29 Transformation involves the uptake, incorporation and expression of free DNA from the environment.
30 Genetic elements such as prophages, transposons or plasmids are not required and natural
31 competence, a developmental stage at which the acceptor strain can take up DNA from the
32 environment and recombine it into its chromosome, is a trait encoded by the acceptor strain. So far,
33 we do not know if transformation may occur frequently in the mammalian intestine. As the gut
34 contains numerous DNA degrading enzymes, it seems likely that the concentration of high-molecular
35 free DNA is low in the gut lumen and thus that transformation is relatively rare in this biosphere.
36 However, stress conditions (as induced by perturbations) have been shown to promote natural
37 competence in bacteria²⁰⁻²².

38 Bacteriophages can ferry genes between bacteria and contribute to HGT in two different ways. They
39 can package and horizontally transfer bacterial genomic DNA between different bacterial species or

1 strains in a process known as transduction. Alternatively, many prophages have incorporated bacterial
2 fitness factors ('morons') within their own genomes. These additional genes are not directly required
3 for the phage lytic cycle (induced, for example, by stress conditions such as host DNA damage^{23, 24})
4 but alter the bacterial phenotype upon integration into the host genome in the form of a prophage²⁵.
5 In contrast to conjugation, this mechanism of HGT does not require direct contact between the donor
6 and the recipient. In fact, as phages are very stable in the environment over extended periods of time,
7 the donor does not even have to reside in the same mammalian host as the recipient. Interestingly,
8 many virulence factors of enteropathogenic bacteria are encoded by prophages^{23, 26}, and prophage
9 integration was found to boost the competitive fitness of the commensal bacterium *Enterococcus*
10 *faecalis*^{27, 28}. In fact, lysogenic prophages integrated in the bacterial chromosome represent the
11 dominant phage form in the intestine²⁹.

12 Intestinal ecosystems harbor a high diversity of conjugative plasmids³⁰. Conjugative plasmids encode
13 the genes required for formation of the conjugation machinery (i.e. the conjugative pilus) which is
14 required for their own transfer. In contrast to transformation and phage-mediated HGT, conjugation
15 requires physical contact of the donor and recipient bacteria³¹. Thus, conjugation is most efficient,
16 when donors and recipients are present at extremely high densities³², as occurs in the intestine (see
17 below).

18 *The intestinal ecosystem: an exquisite playground for HGT.*

19 HGT between the gut microbiota and pathogens can have important consequences for human health,
20 as intestinal bacteria act as reservoirs for fitness factors, virulence genes and antibiotic resistance
21 genes³³⁻³⁵. Why are conditions in the gut especially favorable for HGT? First, the high nutrient inflow
22 and constant temperature allows the maintenance of a continuously active bacterial metabolism.
23 Second, microbial diversity and "amplifier donor strains" (i.e. extremely efficient plasmid donors)
24 may have an "amplification effect" for plasmid transfer³⁶, and host components such as
25 catecholamines can further induce conjugative transfer. For example, norepinephrine at physiological
26 concentrations was shown to enhance conjugative plasmid transfer from a clinical strain of
27 *Salmonella enterica* subspecies *enterica* serovar Typhimurium to an *Escherichia coli* recipient *in vitro*
28³⁷ (see also ref³⁸). [AU: do we know why?]

29 Last, the bacterial population densities are, in general, very high³⁹ and thus conducive for conjugal
30 transfer. Ciliates within the intestine have been shown to increase conjugative plasmid transfer by
31 several orders of magnitude by forming sites of very high bacterial density in their food vacuoles⁴⁰.
32 Likewise, the high bacterial density in the intestine of insects forms a favorable environment for
33 plasmid exchange^{41, 42}. A history of HGT leaves characteristic marks in bacterial genomes as for
34 example shown in the case of the *Bacteroidetes*, which is the most prominent phylum of obligate
35 anaerobic Gram-negative bacteria in mammalian gut ecosystems. A high number of integrative and
36 conjugative elements provide evidence for past HGT events in *Bacteroides* spp.⁴³. One remarkable
37 example for horizontally transferred genes in this genus is the *Bacteriodes fragilis* toxin, which is
38 encoded on a conjugative transposon⁴⁴. Moreover, a recent study identified transposon-associated
39 genes for degradation of algae-derived polysaccharides (for example, from nori, which is used in the
40 Japanese cuisine) encoded by *Bacteriodes* spp. that have been potentially obtained from marine

1 **Bacteroidetes** and are uniquely present in the microbiome of Japanese individuals^{45, 46}. Moreover, it
2 has been shown that intestinal inflammation elicits transient blooms featuring extremely high
3 densities of commensal *Enterobacteriaceae*, in which conjugation-mediated HGT occurs at very high
4 frequency¹⁵. This may explain why conjugative plasmid transfer is particularly pertinent between
5 donor and recipient species that are able to locally bloom under the same environmental conditions.
6 Thus, HGT does occur among the intestinal microbiota and it is further enhanced by blooms.

7 **Blooms, HGT and pathogen evolution**

8 Blooms are formed by bacterial species that are otherwise present in mammalian gut ecosystems at
9 relatively low densities (that is, below 10^8 cfu/ml), for example *Enterococcaceae* and
10 *Enterobacteriaceae* (**Table 1**). Conditions that affect the composition of the microbiota and can thus
11 foster blooms include pathogen infection^{12, 47, 48}, genetic predisposition of the host (for example, in
12 IL010- or TLR5-knockout mice^{49, 50}) and inflammation triggered by colitogenic compounds (for
13 example, dextran sulphate sodium)⁴⁷. Such conditions may foster blooms by increasing high-energy
14 nutrient availability (e.g. Nitrate as a new electron acceptor for anaerobic respiration)⁵¹ or the
15 elimination of competitors that keep colonization levels of bloom-associated species in check in a
16 complex ecosystem (for example, by inducing colonization resistance⁵²).

17 ***Enterobacterial blooms: a hotspot for HGT?***

18 The best-known example of an *Enterobacteriaceae* that can form blooms is *Escherichia coli*, which is
19 usually commensal but under certain conditions can become pathogenic [AU: from below, ok?].
20 Why are these bacteria blooming in the wake of perturbations? *E. coli* is the most abundant facultative
21 anaerobic component of the mammalian gut microbiota but, under homeostatic conditions, represents
22 only a minor fraction of the ecosystem vastly outnumbered by obligate anaerobic bacteria (10^5 - 10^8
23 cfu/g^{52, 53}). Importantly, *E. coli* (like many other *Enterobacteriaceae*) has a very short doubling time
24 and a highly flexible metabolic capacity, including anaerobic respiration of nitrate⁵¹ and a multitude
25 of catabolic pathways which makes it highly adaptable and allows it to bloom in the presence of
26 perturbations⁵⁴. This seems to explain why *E. coli* can exploit situations of reduced colonization
27 resistance and blooms upon disruption of intestinal homeostasis. Such adaptation of *E. coli* to growth
28 in a perturbed gut ecosystem may have opened the door for the acquisition of genetic material by
29 other strains and by pathogens colonizing the gut. Indeed, the high variability in genomic content of
30 *E. coli*, which reflects the high phenotypic diversity between strains, is indicative of constant
31 evolution and diversification. The *E. coli* genome has an open pangenome structure, implying that the
32 species constantly evolves by horizontal gene acquisition and diversification⁵⁵. On average, strain-
33 specific genes that are indicative of horizontal transfer (such as genomic islands, prophages,
34 transposons and plasmids) make up more than 20% of an *E. coli* genome. Furthermore, *E. coli*
35 contains a particularly high diversity of plasmids, which are thought to further enhance mobilization
36 of genetic elements between different strains.

37 Evidence for conjugative transfer to *E. coli* in the context of blooms was recently obtained in the
38 context of infection with the enteropathogen *Salmonella enterica* serovar Typhimurium (*S. Tm*).
39 Infection facilitates not only *S. Tm* growth, but also elicits parallel blooms of commensal

1 *Enterobacteriaceae*, such as *E. coli*. This can fuel the rapid transfer of a conjugative plasmid encoding
2 the bacteriocin colicin from *S. Tm* to intrinsic *E. coli* strains of the normal mouse microbiota¹⁵. Thus,
3 enterobacterial blooms enhance conjugative HGT among pathogenic and commensal members of this
4 family.

5 In addition, phage mediated HGT might also be fuelled, as prophage DNA can represent up to 16% of
6 *E. coli* genome - *Escherichia coli* O157 strain Sakai harbours 18 prophages⁵⁶. As dysbiosis and
7 antibacterial agents can increase phage titers in the gut⁵⁷⁻⁵⁹, it is likely that these increased titers may
8 boost lysogenic conversion of new host bacteria. Thus, in addition to conjugation, perturbation-
9 induced blooms may also boost phage-mediated HGT, on the one hand by lytic induction and by
10 elevating the overall abundance of donors and recipients

11 *Inflammation-induced blooms fostering HGT.*

12 Similarly to *E. coli*, closely related pathogens such as *Salmonella* spp. exhibit a clear history of HGT.
13 During their evolution as pathogens, *Salmonella* spp. have acquired not only their characteristic
14 virulence factors, but also an array of genes favouring growth in the inflamed gut. It has been
15 hypothesized that the evolution of pathogenic *Salmonella* spp. may have happened in two phases
16 (**Figure 2B**; ⁶⁰). In the initial phase, the common ancestor of contemporary *Salmonella* spp. may have
17 acquired genes enhancing growth in the inflamed gut. In this way, this ancestor might have resembled
18 contemporary *E. coli* lineages of the ECOR B2 family, which can bloom in inflamed intestines^{15, 52, 61}.
19 If this were the case, some of these ‘inflammation fitness factors’ should display a history of HGT. An
20 example of this in *E. coli* is the chromosomal *iroBCDEN* gene cluster, which allows bypassing iron-
21 uptake interference by the host protein lipocalin 2⁶². This cluster has been found on transmissible
22 plasmids in uropathogenic *E. coli* strains⁶³, although little is known about the nature of this initial
23 step of pathogen evolution, whether it occurred before or after the divergence from all *E. coli* lineages
24 and which fitness factors had been acquired at this stage. In the second step of pathogenic *Salmonella*
25 spp. evolution, the bacteria would have needed to acquire the capacity to elicit gut inflammation by
26 themselves. Active triggering of gut inflammation has been attributed to a subsequent HGT event,
27 namely the acquisition of the SPI-1 type III secretion system^{60, 64, 65}. Notably, this key virulence factor
28 is encoded by a pathogenicity island with clear evidence of HGT⁶⁶⁻⁶⁸. This enabled *Salmonella* spp. to
29 trigger and sustain mucosal inflammation even when infecting healthy hosts.

30 Most likely, this had a dramatic effect and favoured the acquisition not only of further virulence
31 factors, increasing the efficiency of inflammation, but also of additional fitness factors enhancing
32 growth, survival and transmission in inflamed hosts (**Figure 2B**). In line with this hypothesis,
33 *Salmonella enterica* genomes encode a significant number of prophages, pro-phage remnants, genetic
34 islets and islands as well as plasmids coding for a large array of virulence and fitness factors such as
35 fimbriae (such as *pef*), superoxide-dismutases (such as SodCI and SodIII), LPS (which mediates
36 resistance to bile acid and antimicrobial peptides)^{69, 70} and type III effector proteins^{25, 71}. One example
37 is the SPI-1 TTSS effector protein *sopE*. It is found in just some *S. enterica* strains, including *S.*
38 *enterica* serovar Typhimurium SL1344 and *S. enterica* subspecies *enterica* serovar Typhi, and is
39 encoded on prophages^{23, 72, 73}. Functionally, it enhances host cell invasion⁷⁴ and contributes to gut
40 inflammation^{75, 76}. Furthermore, SopE triggers induction of the host nitric oxide synthetase, which

1 boosts pathogen growth in the intestinal lumen by promoting the conversion of nitric oxide to nitrate
2 (which can be used for nitrate respiration)⁷⁷. Another example is the ability of *S. enterica* subspecies
3 *enterica* serovar Typhimurium to utilize tetrathionate, an electron acceptor generated by oxidative
4 burst of the host inflammatory response⁷⁸. Utilization of this unusual electron acceptor upon
5 anaerobic growth on ethanolamine as carbon source provides a competitive growth advantage of the
6 pathogen over the microbiota in the inflamed gut⁷⁹. Genes required for tetrathionate-respiration are
7 located on the border of *Salmonella* spp. SPI-2. In summary, these examples support the concept of
8 continuing adaptive evolution of *S. enterica* subspecies *enterica* serovar Typhimurium by HGT to
9 efficiently trigger and exploit gut inflammation.

10 In analogy to the continuing evolution of genuine enteric pathogens such as *Salmonella* spp., one may
11 speculate that bacterial adaptation to growth in inflammation-inflicted blooms might foster the
12 emergence of pathobionts (strains adapted to growth in disease-associated blooms by HGT). One
13 might suppose that long-term growth of the microbiota in disease-associated blooms (for example, in
14 inflammatory bowel disease (IBD)) could select for bacteria that are well adapted to withstand the
15 selective pressures imposed by the host's immune response. Resistance to host defences (for example
16 to secretion of defensins, phagocyte killing and iron sequestration) may lead to superior fitness
17 under this condition and therefore become positively selected upon horizontal gene acquisition. As a
18 result, a microbial community enriched in pathobionts may have enhanced disease-promoting
19 potential. Indeed, a microbiota enriched in *Enterobacteriaceae* (*Klebsiella* spp. and *Proteus* spp.)
20 isolated from genetically susceptible colitic mice was capable of driving colitis in genetically intact
21 hosts^{80, 81}. Thus, bacterial blooms occurring as a consequence of intestinal ecosystem perturbations
22 may enhance selection for strains with higher pathogenic potential, e.g. improved capacities to utilize
23 nutrients available in such a disturbed system. The subsequent increase in its population density may
24 foster disease perpetuation. This may feed into a vicious circle of inflammation-induced pathobiont
25 blooms and bloom-induced inflammation (**Figure 3A**). Most likely, additional mechanisms driving
26 such processes will be identified, as we are learning more about the complex microbe-host
27 interactions in the gut ecosystem.

28 ***Bloom-inflicted HGT of antibiotic-resistance ?*** [Au: Please shorten to 45 characters including
29 spaces]

30 The human microbiome harbours a high diversity of antibiotic resistance genes³³. Treatment with
31 antibiotics induces pervasive changes in the composition of the human microbiota and its encoded
32 resistance genes^{8, 82, 83}. In the short-term, the antibiotic transiently decimates the microbiota,
33 compromises colonization resistance and alters homeostasis of the gut-associated immune system^{52,}
34⁸⁴. Interestingly, after a second course of antibiotic therapy, the disturbance of the intestinal ecosystem
35 is less pronounced than what is observed after the first treatment⁸. This suggests that the ecosystem
36 may be able to adapt to the perturbation - for example, by disseminating antibiotic resistances.

37 How could this adaptation be achieved? It is possible that antibiotic-resistance genes encoded on
38 mobile genetic elements (such as plasmids and transposons) could have spread throughout the
39 community, thus conferring resistance to a higher proportion of local strains^{34, 85}. In this case,
40 antibiotic-induced perturbation and concomitant horizontal spread of antibiotic resistance genes

1 would confer increased ecosystem stability. Indeed, it has been reported that the prevalence of
2 antibiotic-resistant strains increased after antibiotic therapy⁸⁶ (**Figure 3B**). However, antibiotic
3 therapy can damage the microbial ecosystem and severely compromise the resilience of the gut
4 microbiota^{87, 88} and increase the risk of infectious disease^{89, 90}.^{14, 48, 89}

5 Pathobionts may also acquire antibiotic resistance genes from commensal bacteria within blooms and
6 may then become positively selected under long-term antibiotic therapy. This condition is of grave
7 concern for human health and of major relevance in clinical settings. A growing number of outbreaks
8 on intensive care units are caused by *Enterobacteriaceae* and Gram-negative non-fermenters like
9 *Pseudomonas* and *Acinetobacter* spp. which are resistant to multiple antibiotics, including all
10 clinically relevant β -lactams. This is mediated by the fast horizontal spread of CTX-M extended-
11 spectrum β -lactamases (ESBLs), plasmid-encoded AmpC β -lactamases and KPC carbapenemases
12 among the *Enterobacteriaceae*⁹¹. However, it remains unclear, if these gene cassettes have originated
13 from commensals, pathobionts or pathogens.

14

15 **Perspective**

16 **[AU: Please add a couple of sentences here summarizing the key points of the article]**

17 The intestinal microbiota is exposed to a number of host- and environmental-derived stressors which
18 affect its functionality by inducing changes in composition and gene expression. Clearly, those factors
19 may also drive evolution of the microbial community over time by positive or negative selection of
20 certain genetic traits. Our current view implies that evolution of certain low-abundance subgroups of
21 the microbiota (e.g. *Enterobacteriaceae*) may not come about at constant rates and much rather
22 happen in ‘hot-spots’ upon microbiota perturbation and dysbiosis: The ability of *Enterobacteriaceae*
23 to grow in ‘blooms’ may provide the basis for efficient genetic exchange by HGT and further
24 adaptation to pathobiont or pathogenic lifestyles.

25 The availability of novel sequencing technologies has opened up unprecedented possibilities to
26 address functional evolution of the microbiota and thus gain a deeper understanding of their flexibility
27 and stability. Now, we have the tools in hand to experimentally address the impact of host and
28 environment-derived factors on the microbiota and its individual members and we can start
29 investigating how these conditions drive short- and long-term microbial genome evolution. So far,
30 metagenomic sequencing approaches have focused on bulk microbiome analysis. Only very recently
31 have studies begun to assemble genomes of individual bacteria from such data⁹². However, this is still
32 quite challenging owing to the short sequence reads and insufficient genome assembly, unless
33 conditions with extremely low diversity are concerned^{7, 93}. Therefore, at the genomic level, it has
34 remained largely unclear to what extent the commensal bacteria in the intestine evolve over time, i.e.
35 by accumulating mutations and by exchanging genetic material via horizontal gene transfer (HGT).
36 The long read lengths of some third-generation sequencing platforms (for example, PacBio and
37 Oxford Nanopore) may greatly enhance the detection of HGT events in microbial genomes even in
38 metagenomic data sets⁹².

1 Most of our current knowledge is derived from studies of enteropathogens and a few cultivable
2 members of the microbiota^{94, 95}. However, an increasing number of reference genome sequences of
3 culturable human and mouse bacterial isolates are being generated (Human Microbiome project;
4 GEBA project). This source of fully assembled genomes from metagenomic data is extremely
5 valuable for analysing genome evolution of commensal bacteria over time, in particular of those
6 species that cannot be cultured *in vitro*^{96, 97}. In addition, genome sequencing of the same strains re-
7 isolated after long-term colonization of the same ecosystem will be important to assess longitudinal
8 genome stability of different bacterial types over time and to identify the confounding environmental
9 variables. Recently, several studies shed light on a new aspect of bacterial genome diversification in
10 the intestine: upon colonization of the gut by a single *E. coli* clone, different *E. coli* mutants that
11 stably co-colonized the gut over long term were positively selected for according to increased stress
12 resistance and nutritional competence^{98, 99}. These data indicate a “sympatric diversification” into
13 functionally diverse mutants that can exploit different nutritional or stress-imposed niches within the
14 same gut ecosystem (known as the ‘restaurant hypothesis’⁹⁹). This possibility will further complicate
15 the analysis: processes such as sympatric diversification among the gut microbiota pose extreme
16 challenges to experimental and analytical systems which may be eventually overcome by genome
17 sequencing strategies targeting individual bacterial cells retrieved from the microbiota and thereby
18 allow detecting single nucleotide polymorphisms which may underlie such diversification.[Clearly,
19 key discoveries on microbiota function in health and disease will rely on additional technological
20 advances in DNA sequencing, and single cell analysis¹⁰⁰.

21 **Acknowledgements**

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23 lab of BS is supported by the DFG and the Federal Ministry of Education and Research (BMBF).

24

1 Box 1: Dysbiosis

2

3 Early in the 20th century, the Russian Nobel prize laureate Elie Metchnikoff introduced the term
4 dysbiosis (also known as dysbacteriosis) [AU: ok?] to describe the opposite of symbiosis - a state of
5 co-existence in mutual harmony ¹⁰¹. A dysbiotic microbiota therefore is an imbalanced intestinal
6 microbial community (including bacteria, yeast, viruses and parasites) characterized by quantitative
7 and qualitative changes in the [Au: composition of?] microbiota itself, its modified metabolic
8 activities or changes in local distribution of its members. [AU: moved from below] During a state of
9 dysbiosis, the microbiota is prone to invasion and blooms of pathogens exploiting niches left open
10 after disturbances. Dysbiosis is a potential trigger of disease and is commonly associated with
11 different diseases ranging from diarrhea and constipation to IBD ¹⁰², obesity ¹⁰³, cancer ¹⁰⁴, diabetes ¹⁰⁵
12 , allergy and asthma ¹⁰⁶. Most of these diseases involve inflammation, which itself is often caused by
13 altered immune responses to intestinal bacteria. As such, immune dysregulation and gut dysbiosis
14 frequently coincide and can occur as a result of one another.

15

16

1

2

1 **Figure legends**

2 **Figure 1: Functional classification of the members of the gut ecosystem..** Symbiosis describes a
3 prolonged and close association between two species resulting in a benefit for at least one of these
4 organisms. Symbiosis can be further subdivided into mutualism (right; each member benefits),
5 commensalism (middle; only one member benefits, the other is unaffected) and parasitism (left; one
6 organism benefits at the expense of the other). The intestinal microbiota is characterized by both
7 commensal and mutualistic properties. Enteric pathogens including obligate and opportunistic
8 pathogens lead a parasitic lifestyle. Colonization with an obligate pathogen leads to disease outcome
9 (i.e. *Vibrio cholerae*, *Shigella* spp., *Salmonella* spp.). Opportunists colonize their host only under
10 favorable conditions (i.e. immunosuppression; *Legionella pneumophila*, *Pseudomonas aeruginosa*) or
11 show their pathogenic potential only under specific circumstances, such as during dysbiosis
12 (=pathobionts; *Clostridium difficile*, vancomycin-resistant *Enterococcus* (VRE)). Low-abundance
13 symbionts with the ability to destabilize a homeostatic microbiota towards a dysbiotic state are
14 referred to as keystone pathogens (*Porphyromonas gingivalis*). Here, in contrast to pathobionts, an
15 already imbalanced ecosystem is not a prerequisite. Symbionts with the opposite effect are referred to
16 as keystone stabilizers (i.e. *Bacteroides thetaiotaomicron*)¹⁰⁷. The blue gradient indicates the risk for
17 bacterial blooms during a given condition.

18

19 **Figure 2. Mechanisms of HGT and enteric pathogen evolution. A.** Mechanisms of HGT. For each
20 type of HGT, it is indicated whether it is dependent on bacterial blooms and whether it has been
21 shown to occur in the gut. **B.** Two-stage model for the evolution of the enteric pathogen *Salmonella*
22 spp.. In a first step, the common ancestor of commensal *E. coli* strains (i.e. ECOR B2) and
23 contemporary *Salmonella* spp. may have acquired genes enabling growth in the inflamed intestine
24 (e.g. genes for iron acquisition). While commensal *E. coli* remained at this level, *Salmonella* spp.
25 acquired virulence factors (i.e. pathogenicity islands 1, 2 and 4) in the next stage to trigger gut
26 inflammation itself. From stage two, horizontal acquisition of fitness- and virulence factors has been
27 promoted by growth within inflammation-induced blooms. **[Au: Please add complete legend,**
28 **describing each part of the figure sequentially]**

29

30 **Figure 3. Perturbation-induced destabilization and stabilization of intestinal ecosystems. A.**
31 Perturbation-induced destabilization of the gut ecosystem. Perturbation-induced blooms can lead to
32 dysbiosis and positive selection of pathobionts. Bacteria may adapt to growth in dysbiotic conditions
33 and acquire even higher pathogenic potential by horizontal spread of virulence-factors. This process
34 may end up in a vicious cycle in which perturbation-induced blooms increase inflammation in turn
35 promoting pathobiont blooms. **B.** Perturbation-induced gut ecosystem stability. Perturbations may
36 imply the spread of genes among the intestinal microbiota which confer resistance to the perturbation
37 itself. This may be the case upon antibiotic treatment and thereby positive selection for horizontally

- 1 transferred antibiotic-resistance genes. After resilience, the evolved ecosystem will be more resistant
- 2 to a second exposure to the same antibiotic.
- 3

1 **Tables**

2 **Table 1:** Studies reporting blooms of particular bacterial species in response to environmental
 3 changes or host-imposed perturbations [AU: The text within the table is a little to long, so
 4 shortened to ensure that the table fits on one page. Also, we have added a new column to clarify
 5 the relevance of the table to HGT and the rest of the article]

Nutrient-induced blooms				
<i>Ref.</i>	<i>Species</i>	<i>Bloom characteristic</i>	<i>Function relevant for blooming</i>	<i>Evidence of HGT^s</i>
45	<i>Bacteroides plebeius</i>	Enrichment of <i>Bacteroides</i> spp. with metabolic capacity to degrade algae-derived polysaccharides	Porphyrinases, etc. derived from marine bacteria	Genes are transposon-associated
108	<i>Bilophila wadsworthia</i>	Consumption of a diet high in saturated fat promotes the expansion of a low-abundance, sulphite-reducing pathobiont	Sulfite reduction	No
Antibiotic-induced blooms				
90	<i>E. coli</i> pathobiont	Multidrug-resistant blooms noted in antibiotic-treated mice and caused a sepsis-like disease via Naip-5-Nlrc4 inflammasome activation.	AB resistance genes (i.e. against ampicillin, neomycin)	Unknown
88	<i>Enterobacteriaceae</i>	One dose of clindamycin promotes enterobacterial blooms and contractions of other bacterial taxa in mice, enhancing susceptibility to <i>Clostridium difficile</i> induced colitis	Unknown	Unknown
89	<i>Enterococcus</i> spp., <i>Streptococcus</i> spp., γ - <i>Proteobacteria</i>	Intestinal blooms noted during allogeneic HSC transplantation [AU: are these relevant to antibiotics? Or immunosuppressants?]	Unknown	Unknown
Inflammation-induced blooms				
109	Symbiotic <i>E. coli</i> NC101	Mucosa-associated strains increase in IBD and colorectal carcinoma (CRC) patients; monocolonization in CRC <i>III0</i> ^{-/-} mouse model promotes invasive carcinoma	polyketide synthase (<i>pks</i>) genotoxic island	Gene encoded on genotoxic island

15	Symbiotic <i>E. coli</i> and <i>Salmonella</i> Typhimurium	Co-blooms in a mouse colitis model boosted conjugative horizontal gene transfer, which, under non-inflammatory conditions, was inhibited by the microbiota	Conjugative colicin plasmid	Gene encoded in plasmid
35, 62, 78, 23, 77	<i>Salmonella</i> Typhimurium <i>Citrobacter rodentium</i> <i>Campylobacter jejuni</i> <i>Enterobacteriaceae</i>	Inflammation shifts the balance between protective microbiota and the pathogen, promoting pathogen bloom	<ul style="list-style-type: none"> • <i>iroBCDEN</i> cluster • <i>ttrs</i>-cluster • <i>sopE</i>-phi 	Gene encoded in genomic islands
110	Symbiotic <i>E. coli</i> [AU: is the bloom of <i>E. coli</i> or <i>C. jejuni</i> ?]	Blooms in infant mice susceptible to <i>C. jejuni</i> and colitic mice. Artificial modification of the microbiota by feeding live commensal <i>E. coli</i> ; increased susceptibility to <i>C. jejuni</i> in healthy mice	unknown	unknown
Immune deficiency or host genetics factors resulting in blooms				
50	<i>Proteobacteria</i>	TLR-5 deficient mice show transient microbiota instability characterized by high levels of proteobacteria, encouraging inflammation.	unknown	unknown
Disease-dependent blooms				
111	Adherent and invasive <i>E. coli</i> (AIEC)	Higher prevalence within the ileal mucosa in Crohn's disease patients	unknown	unknown
112	<i>Helicobacter hepaticus</i>	T6SS mutants elicit increased inflammation in an experimental model of colitis (T cell transfer in Rag1 ^{-/-} mice)	Absence of T6SS	unknown
Neonatal colonization				

113-115	Facultative anaerobes	Infants harbour increased levels of facultative anaerobic microorganisms and reduced levels of strict anaerobes. Such communities are intrinsically unstable and highly susceptible to interference by stressors and infections with opportunistic pathogens	unknown
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