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Bacterial pathogenesis as an imperfect symbiosis

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Summary. Human-adapted bacterial pathogens such as *Salmonella enterica* serovar Typhi, *Helicobacter pylori*, and *Mycobacterium tuberculosis* cause acute infections and also latent, asymptomatic infections. During latent infection the pathogen undergoes self-attenuation of virulence, a lifestyle that reduces the impact of infection on host fitness. Evolutionary strategies of this kind may drive certain bacterial pathogens towards commensalism.

Keywords: evolution · human pathogens · host susceptibility · symbiosis

Resum. Els patogens bacterians adaptats als humans com ara *Salmonella enterica* serovar Typhi, *Helicobacter pylori* o *Mycobacterium tuberculosis* causen infeccions agudes i també infeccions latents i asimptomàtiques. Durant una infecció latent, el patogen atenua la seva virulència i adopta un estil de vida que redueix l'impacte de la infecció sobre el benestar de l'hoste. Estratègies evolutives d'aquest tipus poden dirigir alguns patògens bacterians vers el comensalisme.

Paraules clau: evolució · patògens humans · susceptibilitat de l'hoste · simbiosi

Popular culture views bacteria as enemies of humans, and an example is found in a monosyllabic, euphonic poem by Catalan writer Joan Oliver (aka Pere Quart):

"BACIL
Ni
bri
bo:
microbi."

("BACILLUS: Not good at all: microbe") [36]. Popular aversion for bacteria is radically opposite to scientific knowledge. Biogeochemical cycles are made possible by bacterial metabolism, which recycles molecules and chemical elements to make life sustainable. Our body is also the home for thousands of bacterial species, and the total number of bacterial cells in a healthy human may be similar (or even exceed) the number of "human" cells: between 10^{12} and 10^{13} [44]. Large bacterial communities are present in a variety of organs (Table 1). These bacteria are part of the so-called normal microbiota, and establish commensal or mutualistic relationships with us. Bacterial contributions to human health include synthesis of vitamins, detoxification of dangerous compounds, adjustment of the immune system, and protection against pathogenic microorganisms by direct competition [44].

Compared with the thousands of bacterial species that are either innocuous or beneficial for us, pathogenic bacteria are rare. Furthermore, their involvement in disease is complex, and the view of infectious disease as the attack of one organism by another organism is often naive. Bacteria that cause disease can be classified in two types. A few species are primary ("true") pathogens. Many others, however, are opportunistic pathogens that cause disease in specific circumstances only [42]. Environmental, usually harmless species can cause disease when a physiological alteration occurs

(e.g., a burn or an impairment of innate immunity). Other opportunistic pathogens are commensals of the human body, and live in an equilibrium sustained by multiple factors. When the equilibrium is disrupted, the bacterial population proliferates and causes disease, sometimes colonizing organs where the microorganism is not normally found [42].

The distinction between primary and opportunistic pathogens is not absolute: upon infection by a primary pathogen, certain individuals can be spared from disease by genetic or physiological factors. A risky experiment showing that an encounter with a primary pathogen does not necessarily lead to disease was performed by German microbiologist Max von Pettenkoffer in the 19th century. As part of his polemics with Robert Koch on the ethiology of cholera, Max von Pettenkoffer drank a *Vibrio cholerae* preparation without suffering contagion [30]. It is also well known that epidemics affect certain members of a population while others, sometimes in the same family, do not suffer the disease. Genetic polymorphism may be a major cause of individual differences in susceptibility to infection, as predicted by the Red Queen hypothesis: because pathogens prey on the most common host genotypes, a selective advantage for rare host genotypes is generated [20]. However, nutritional and physiological factors have also a major impact on host susceptibility to infection.

Table 1. Mass of bacterial communities in human organs*

Organ/ system	Weight of bacterial mass (grams, net weight)	Number of bacterial cells
Eyes	1	10^9–10^{10}
Nose	10	10^{10}
Mouth	20	10^9–10^{11}
Lungs	20	10^{10}
Vagina	20	10^{10}
Skin	200	10^{10}–10^{11}
Intestine	1000	10^{12}

*Adapted from [44].

Evolutionary strategies of pathogens

Despite the multiplicity of factors involved in host-microbe interactions, reductionist modeling of host-pathogen interactions is possible. While the Red Queen hypothesis addresses the impact of infection on host evolution, classical studies by Robert M. May have modeled the evolution of virulence in pathogens. A major question, for instance, is whether pathogens that evolve to be harmless to their hosts do better than those that do not attenuate their virulence. A useful measure of pathogen fitness is the reproductive rate, which can be defined as the product of two independent factors: the rate of infection of new hosts and the time of host survival after infection [27]. Theoretical analysis of reproductive rates predicts that evolutionary success can be achieved in many manners, and that co-evolution of hosts and parasites can follow different paths. If new hosts are efficiently infected, host survival will matter little. However, because the number of hosts cannot be infinite, high infection rates do not warrant long-term success. In fact, lowered levels of host fitness can compromise survival of the pathogen if the number of hosts decreases below a critical level.

Pathogens that do not attain high rates of infection can benefit if the fitness of their hosts is not severely impaired upon infection. The longer the host lifespan, the higher the reproductive rate. While many viruses (not all) behave as "rogue" parasites that increase their reproductive rate at the expense of host fitness, many bacterial pathogens seem to increase their reproductive rates by reducing their virulence. Self-attenuation of virulence is not only observed in opportunistic pathogens, which cause disease by accident. The evolutionary strategies of primary pathogens also show signs of self-attenuation, especially in bacterial species that colonize one or a few eukaryotic hosts. In fact, host adaptation and attenuation of virulence seem to be related evolutionary processes. This kind of evolution converts infectious disease into a sort of imperfect symbiosis. This article is mainly centered on one primary pathogen, *Salmonella enterica*, which presents unequivocal signs of self-restraint during infection of animals. Other primary pathogens such as *Brucella*, *Chlamydia*, *Helicobacter*, *Staphylococcus,* and *Mycobacterium* are also known to undergo self-restraint upon infection [38].

An example of primary pathogen: *Salmonella*

Salmonella enterica is a pathogenic relative of the human commensal *Escherichia coli*. Although the evolutionary divergence between the genera *Escherichia* and *Salmonella* may have occurred 150 million years ago, their chromosomes remain similar [18]. A relevant difference, however, is the presence of several pathogenicity islands in the *Salmonella* genome [18]. Genes contained in pathogenicity islands encode type III secretion systems and effector proteins involved in host colonization.

The currenty accepted taxonomy divides the genus *Salmonella* in species, subspecies and serovars. *S. enterica* subspecies *enterica* includes most of the serovars that cause disease in humans and livestock animals. Serovars Typhimurium, Enteriditis, and Typhi are frequently isolated from humans, and they are associated with an estimated 115 million clinical cases per year worldwide [11,37]. Depending on the serovar and the host, *Salmonella* infections of healthy (immunocompetent) humans can be classified into several types. One is gastroenteritis, leading to diar-

rhea and inflammation [37]. Gastroenteritis is a mild infection, extremely common in developed countries, and involves self-limited infection of the terminal ileum and colon. In contrast, typhoid fever is a life-threatening infection in which *Salmonella* disseminates through the lymphatic system and is transported within phagocytes [11]. Dissemination permits colonization of the liver, the spleen, the bone marrow, and the gall bladder. Death may occur as the consequence of the phenomenon known as septic shock, an overeaction of the host immune system triggered by *Salmonella* lipopolysaccharide [43]. In the twentieth century, typhoid fever was virtually eradicated in developed countries by the development of drinking water treatment, water sewage control, pasteurization of milk and dairy products, and other food technology practices. However, typhoid fever remains a major problem for public health in developing countries: around 25-30 million cases are estimated to occur per year worldwide [11].

A fraction of individuals recovering from typhoid fever become asymptomatic, life-long carriers of *Salmonella enterica* ser. Typhi [11]. Non-typhoidal *Salmonella* serovars can also cause persistent infections, either associated with cholecystitis or asymptomatic, although the duration of carriage is usually limited to several months [37]. Persistent and chronic infections, especially if asymptomatic, may be viewed as evolutionary strategies that increase the *Salmonella* reproductive rate by favoring host survival. Like humans, livestock and wild animals can be asymptomatic carriers of non-typhoidal *Salmonella* serovars able to infect humans, thus acting as reservoirs for human infection (Fig. 1).

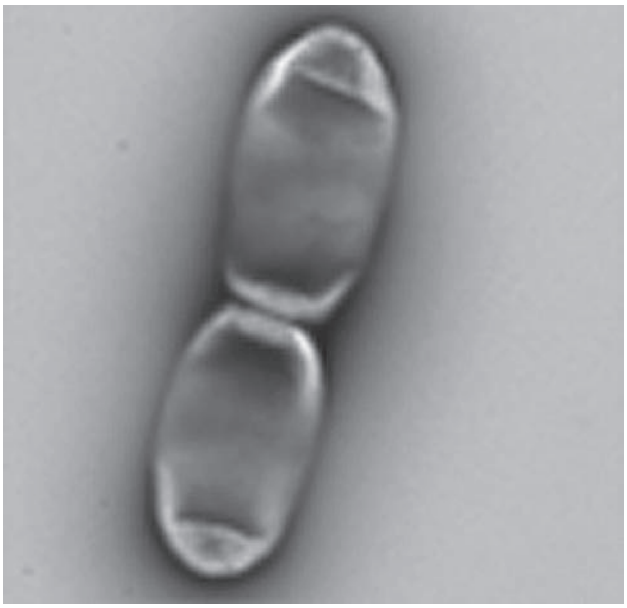

Fig. 1. A *Salmonella enterica* cell dividing in the presence of 5 % sodium deoxycholate. Survival and division in the presence of bile salts is made possible by activation of bile resistance responses [22]. (Electron microscopy image obtained by Miguel A. de Pedro, Severo Ochoa Molecular Biology Centre (CBMSO), Cantoblanco, Spain).

Chronic carriage of *Salmonella*

Human carriage of *Salmonella* Typhi is often asymptomatic, and therefore difficult to diagnose. At the beginning of the twentieth century, a famous case was protagonized by Mary Mallon, the first chronic carrier identified in the United States. Mallon, an Irish immigrant, is presumed to have infected at least 51 people, three of whom died, over the course of her career as a cook in New York [5].

Antibiotics are usually ineffective on *Salmonella* carriage (even if Salmonellae are susceptible to them) because *Salmonella* resides in the gall bladder [17]. Bacterial proliferation in the gall bladder of chronic carriers permits *Salmonella* shedding to the environment, potentially transmitting the infection.

The ability of *Salmonella* to thrive in the human gall bladder is one of most surprising aspects of *Salmonella* biology because the high concentration of bile present in the gall bladder makes it a harsh environment for bacteria: bile salts disrupt the bacterial envelope, denature proteins, and damage DNA [4,8,29]. However, *Salmonella* escapes the bactericidal action of bile salts. Current evidence suggests that *Salmonella* resistance to bile does not involve a single response but a variety of responses. It seems also likely that *Salmonella* subpopulations are formed upon gall bladder colonization, and that these subpopulations respond to bile in different ways [3]. A fraction of the *Salmonella* population invades the epithelium, where it may be protected, at least partially, from bile salts [28]. Another subpopulation multiplies in the gall bladder lumen, either forming biofilms on gallstones or activating bile defense responses [10,19]. Biofilm formation not only contributes to bile resistance but also to antibiotic resistance, thereby explaining why patients carrying *S.* Typhi in the gall bladder usually fail to respond to antibiotic treatment [17]. In turn, planktonic *Salmonella* cells survive in the gall bladder by activating bile defense responses. *In vitro* studies suggest that bile defense may combine activation of efflux pumps, remodeling of the bacterial envelope, and activation of stress responses and DNA repair functions [22,33-35]. Adoption of multiple lifestyles in the gall bladder may help to understand the difficulty to eradicate *Salmonella* chronic carriage, which often makes cholecystectomy the only effective treatment.

Stealthy *Salmonella* persistence inside fibroblasts

The possibility that the gall bladder is not the only reservoir for *Salmonella* Typhi during persistent and chronic infections has been considered for decades, and has been re-ex-

amined recently [31]. It seems possible, for instance, that *Salmonella* Typhi may invade cell types other than its classical cell targets (epithelial cells, dendritic cells and macrophages), perhaps adopting a latent intracellular lifestyle. In the mouse model of typhoid fever, *Salmonella* Typhimurium can invade fibroblasts, a ubiquitous cell type with long lifespan in connective tissues [38]. Interestingly, colonization of fibroblasts by *S. Typhimurium* is not followed by bacterial proliferation. This restrained lifestyle resembles the persistent state which in other microorganisms is known as dormancy [16]. Persistence of *Salmonella* inside fibroblasts is an example of bacterial self-restraint, as indicated by the isolation of *S. Typhimurium* mutants able to proliferate inside fibroblasts [6]. The bacterial functions that restrain growth inside fibroblasts are also required for virulence, and overgrowth inside fibroblasts does not cause hypervirulence but loss of virulence [15]. Hence, a tentative link can be established between successful infection and bacterial self-restraint. The peculiar nature of the *Salmonella*-fibroblast interaction is further illustrated by the fact that invasion of fibroblast cell lines differs from entry into epithelial cells, especially in the way the bacterium induces rearrangement of the eukaryotic cytoskeleton [1]. Collectively, these findings support the possibility that *Salmonella*, like other intracellular pathogens, may have evolved strategies to avoid host damage and to favor stealthy bacterial persistence in host tissues. On the other hand, slow-growing *S.* Typhimurium variants have been isolated upon long-term passage through fibroblasts [7], and these mutants resemble the *Staphylococcus aureus* small colony variants that cause chronic infections [41] (Fig. 2).

Attenuation of *Salmonella* virulence by subpopulation formation

Single cell analysis technologies (e.g., flow cytometry and microfluidics) reveal that phenotypic heterogeneity is common in bacterial populations made of genetically identical cells [12]. In some cases, formation of bacterial subpopulations is programmed by genetic or epigenetic mechanisms; in other cases, phenotypic heterogeneity is the consequence of noisy gene expression. Noise can be used also as a signal to trigger an epigenetic feedback loop when gene expression reaches a threshold [26]. Because this threshold is only reached in certain cells, the bacterial population splits into two cell types with distinct properties.

Subpopulation formation is observed at several stages of *Salmonella* infection, and may contribute to virulence attenuation. For instance, synthesis and secretion of the *Salmonella* effectors necessary for epithelial cell invasion is restricted to a fraction of the bacterial population [2]. *Salmonella* entry into macrophages is also accompanied by formation of two subpopulations, one of which replicates while the other enters a dormant-like state [21]. Like other bacterial species, *Salmonella* also presents phenotypic heterogeneity generated by phase variation, the reversible switch of gene expression at high frequency (e.g., $>10^{-5}$ per cell and generation) [39,40]. Switching turns gene expression from OFF to ON, or from low expression to high expression, and *vice versa*. An example is the *opvAB* locus of *Salmonella* which controls O-antigen chain length in the lipopolysaccharide [9]. Phase variation of *opvAB* generates two subpopulations, one with long O-antigen chains, another with shorter O-antigen chains.

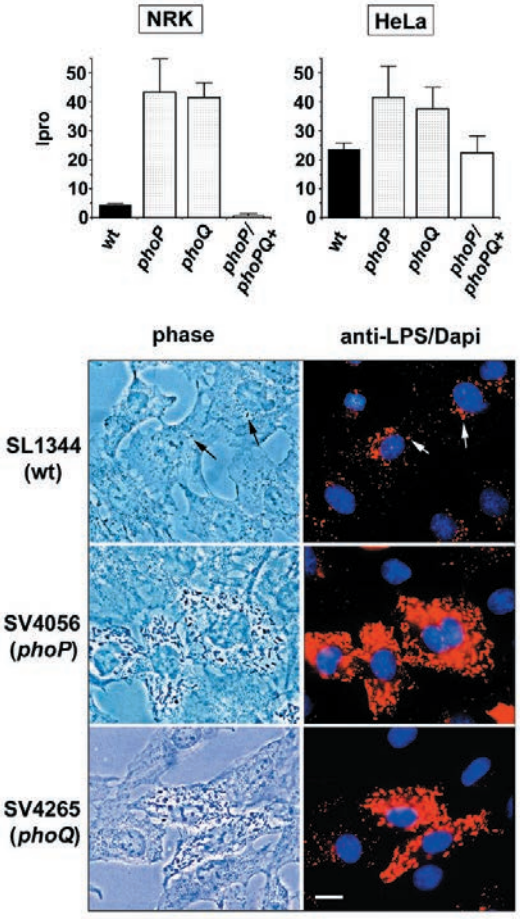

Fig. 2. Overgrowth of *Salmonella enterica phoPQ* mutants within eukaryotic cells. A. Intracellular growth rates (*Ipro* values) of wild type *S. enterica* (SL1344), a *phoP* mutant (SV4056), and a ***phoQ*** mutant (SV4365) in NRK fibroblasts and HeLa epithelial cells. Overgrowth is observed in fibroblasts and to a lesser extent in epithelial cells. B. Massive overgrowth of *Salmonella phoP* and *phoQ* mutants in NRK fibroblasts as observed by immunofluorescence microscopy. Monoclonal mouse IgG anti-LPS antibody and goat anti-mouse IgG conjugated to Texas Red were used for bacterial staining (red). DAPI (4,6-diamidino-2-phenyl-indole) was used to stain eukaryotic nuclei (blue). Arrows indicate wild type bacteria that do not exhibit massive intracellular proliferation. Bar: 10 µm. (Reproduced from [6], © American Society for Microbiology, Infect. Immun. October 2001; doi:10.1128/IAI.69.10.6463-6474.2001).

These subpopulations differ in their capacity to invade macrophages and in their resistance to serum; as a consequence, one subpopulation is more virulent than the other [9]. Other *Salmonella* loci that show phase variation are the *pef* and *std* operons, which encode fimbriae for attachment to specific host tissues [23,32]. Attenuation of virulence by subpopulation formation may benefit the pathogen by escaping the immune system and other host defense mechanisms. However, it can also benefit the host by reducing the burden of bacterial infection (Fig. 3).

Chronic infection as an imperfect symbiosis

The ability of *Salmonella* Typhi to cause chronic infection may be viewed as a rudimentary symbiosis: the presence of *Salmonella* in the gall bladder does not cause clinical symptoms in most human carriers. The symbiosis is however imperfect because colonization of the gall bladder is not completely harmless to the host: gallstone formation is a risk factor for the development of hepatobiliary cancer, probably in combination with genetic predisposition and other risk factors [13].

Besides *Salmonella* Typhi, other primary pathogens establish latent infections that may be viewed as imperfect symbioses. *Helicobacter pylori*, for instance, causes asymptomatic infection in over 80 % of humans [24]. Latent infection by *Helicobacter* resembles *Salmonella* chronic carriage in that both infections occur in hostile environments: the bile-laden gall bladder for *Salmonella*, the acid environment of the stomach for *Helicobacter*. An additional analogy is that in both cases latent infection permits shedding of bacteria and propagation to new hosts. Although the presence of *Helicobacter* in the stomach can cause ulcers and gastric cancer in certain individuals, epidemiologic studies suggest that latent infection with *Helicobacter* can protect against esophageal cancer [14].

Mycobacterium tuberculosis, the causal agent of tuberculosis, is able to persist within humans for long periods without causing clinical symptoms of disease [25]. In Europe and the U.S., carriage affects at least 1% of the population, with enormous regional differences. Chronic persistence of *Mycobacterium* within the human host involves a state known as dormancy: bacterial metabolism is slowed down and cell division is arrested [16]. Dormancy occurs in the vacuole of infected macrophages. Intracellular location of the pathogen, metabolic slowdown and cell division arrest make pharmacological treatment of dormancy useless. Unlike chronic infection by *Salmonella* or *Helicobacter*, latent carriers of *M. tuberculosis* do not shed bacterial cells into the environment.

The view that primary pathogens and commensals are separated by a blurred line is further supported by epidemiological evidence suggesting that reduction of human exposure to infectious agents may have increased allergies and autoimmune diseases [14]. In fact, a widely accepted notion is that interaction with infectious agents, especially during infancy and childhood, may play a key role in development and tuning of the human immune system. If latent infections by human-adapted pathogens play beneficial protective roles, acute disease may be considered an accident triggered by factors that disrupt the host-pathogen equilibrium. As pointed out by Stanley Falkow, organisms like *Helicobacter pylori*, the typhoid bacillus, and *Mycobacterium tuberculosis* have been our constant companions through human evolution, and might be considered part of the normal human flora along with the accepted commensals [14].

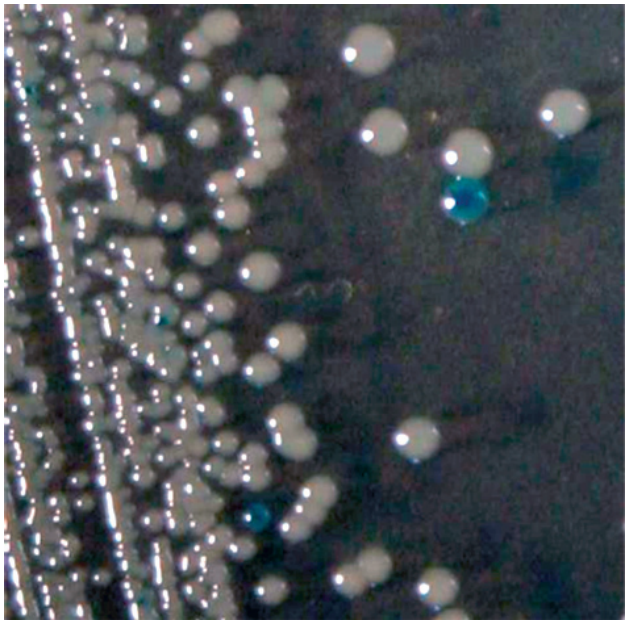

Fig. 3. Phase variation of the *Salmonella enterica opvAB* operon as observed in bacterial colonies. The ***Salmonella*** strain carries a *lacZ* fusion in the *opvAB* operon. Expression of *opvAB::lac* gives rise to blue colonies on LB agar containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Cells that do not express ***opvAB*** form white colonies on LB containing X-gal. (Reproduced from [9], Creative Commons License).

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