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Minireview

Bacterial pathogens: from natural ecosystems to human hosts

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Summary

The analysis of the genomes of bacterial pathogens indicates that they have acquired their pathogenic capability by incorporating different genetic elements through horizontal gene transfer. The ancestors of virulent bacteria, as well as the origin of virulence determinants, lay most likely in the environmental microbiota. Studying the role that these determinants may have in non-clinical ecosystems is thus of value for understanding in detail the evolution and the ecology of bacterial pathogens. In this article, I propose that classical virulence determinants might be relevant for basic metabolic processes (for instance iron-uptake systems) or in modulating prey/predator relationships (toxins) in natural, non-infective ecosystems. The different role that horizontal gene transfer and mutation may have in the evolution of bacterial pathogens either for their speciation or in short-sighted evolution processes is also discussed.

Introduction

'To pathogenic microparasites (viruses, bacteria, protozoa, or fungi), we and other mammals (living organisms at large) are little more than soft, thin-walled flasks of culture media.' This sentence, taken from an article by Levin and Antia (2001), reflects in my personal view the basic ecological reasons of the evolution of microbial pathogens. These reasons are the benefits obtained by an organism when it occupies a novel environment (in this case a human host) and the elements needed for such occupation. Following Levin's reasoning a little further, for bacteria, the human body is a rather rich habitat composed by different micro-environments, which altogether contain a large variety of nutrients (Eisenreich *et al.*, 2010). The human host can be considered as well an extreme ecosystem, since its infection is precluded by the host defence mechanisms. Gaining access to this nutrient-rich habitat is of course beneficial for a bacterium and for this task two types of elements are needed. (i) Determinants required for avoiding the defence mechanisms of the host. These mechanisms include elements developed by the host itself as the immune system, the stomach pH barrier or the detergent activity of bile salts. Other elements that impede infection and are not the result of human evolution are the anti-colonization activity of human microbiota and, more recently, the use of antibiotics (Martinez and Baquero, 2002). (ii) Elements of the bacterial metabolism required for growing in the infected host. Obviously, an organism unable to grow at 37°C, at the oxygen tension or at the osmolarity present in human tissues, will be unable to produce an infection. Similarly, an organism that does not harbour an efficient iron-uptake system, will rarely produce infections in a habitat, such as the human body, where iron availability is scarce (de Lorenzo and Martinez, 1988; Martinez *et al.*, 1990). In occasions, some elements may be involved in both aspects of infection. For instance, proteases can be useful for disrupting the extracellular matrix or to degrade proteins involved in defence against infection (Galloway, 1991), but they serve as well for providing amino acids and peptides that are useful nutrients.

If infection is just a way of gaining access to a new (extreme) habitat, how bacteria have acquired the tricks needed for this process? Like in any event of bacterial evolution, there are two ways for this process: acquisition of genes by horizontal gene transfer (HGT), and mutation (Boto and Martinez, 2011). Indeed, it is well known that several bacterial pathogens have evolved trough the acquisition of pathogenicity islands (Groisman and Ochman, 1996; Morschhauser *et al.*, 2000; Hacker and Carniel, 2001; Schmidt and Hensel, 2004) and that mutation is highly important for the evolution of pathogens producing chronic infections (Oliver *et al.*, 2000; Martinez-Solano *et al.*, 2008). However, one aspect that is not so frequently discussed concerns the origin of pathogenicity islands and the functions that these elements might have

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in their original hosts, before their transfer to the pathogen. It can be thought that the human commensal microbiota can be a source of at least some of the elements that bacterial pathogens require for growing inside the human host. However, there is no evidence of such situation. It might be also that pathogenic bacteria were originated in animals and indeed some works indicate that this situation might have happened at the Neolithic age with the establishment of agriculture and animal domestication (Pearce-Duvet, 2006; Wolfe *et al*., 2007). However, even in these cases, animals' pathogens harbour pathogenicity islands that must have been originated in another organism. In this review I will discuss the different roles that HGT and mutation might have for the evolution of bacterial pathogens and will present some ideas on the potential roles that the elements present in pathogenicity islands might have in non-clinical ecosystems.

From the host's point of view, colonization and infection are different processes. However, from an ecological viewpoint, gaining access to a new habitat can be considered as its colonization, irrespectively on which is the impact of the entrance of the microorganism for this environment. Indeed, the term infection is not used unless a living host is the colonized habitat. Because of this, a clear cut-off between infection and colonization will not be made along the review unless the process is being discussed under the host's viewpoint.

Causes and consequences of gaining access to a new ecosystem

As stated above, the human body can be considered as an extreme ecosystem for an environmental microorganism, because it has developed sophisticated systems for avoiding infections. Gaining access to this extreme habitat requires the acquisition in a single step of different elements that serve to resist and subvert the activity of the human defence systems and eventually to gain the metabolic capabilities required for growing inside the host. For simple organisms as viruses, mutation and recombination can render a change in a receptor binding protein that allows a host change (Domingo, 2010; Pepin *et al*., 2010; Li *et al*., 2011; Mehle *et al*., 2012). For bacteria the situation is more complex and mutation-driven evolution cannot justify by itself the acquisition at the same time of the several traits required for producing an infection. The inspection of the genomes of bacterial pathogens reveals this is achieved by acquisition, through HGT of genetic elements, including pathogenicity islands and plasmids (Hacker *et al*., 1997; Schmidt and Hensel, 2004; Ahmed *et al*., 2008; Juhas *et al*., 2009). If virulence is acquired in *quantum leaps* through the incorporation into the genome of a non-virulent organism of a set of novel genes, this will be reflected by the clonal expansion of the strain that has acquired these traits. Indeed, pathogenic bacteria as *Bacillus antracis*, *Yersinia pestis* or *Francisella tularensis*, are examples of the recent expansion of fit clones (Keim and Wagner, 2009) that have acquired specific genetic elements that allow the colonization of new hosts (animals and humans). As discussed below, this does not mean that mutation and gene loss are of no relevance for the evolution of pathogens; only that gene acquisition is frequently a first event in this process.

One example that has been studied in detail is the evolution of the cause of plague, *Y. pestis* (Achtman *et al*., 2004; Zhou and Yang, 2009; Morelli *et al*., 2010). There have been three plague pandemics, which together have produced around 200 millions deaths at historical times when human population was much lower than it is today. The genus *Yersinia* is formed by 15 species, and only three of them, namely *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*, are pathogenic to humans. Phylogenetic analyses have shown that *Y. pestis* and *Y. pseudotuberculosis* diverged from *Y. enterocolitica* more than 40 million years ago. *Yersinia pestis* is a clone that derived from the enteric pathogen *Y. pseudotuberculosis* less than 20000 years ago (Achtman *et al*., 1999). The successful expansion of this newborn *Y. pestis* clone was caused by the incorporation into the genome of *Y. pseudotuberculosis* of a group of genes that allow *Y. pestis* to make use of a different route of transmission and produce a different type of infectious disease. *Yersinia pseudotuberculosis* is a food-borne pathogen that usually produces non-fatal gastrointestinal diseases. *Yersinia pestis* transmission is caused by inhalation or by the bite of an infected flea and has been the cause of septicaemic, pneumonic and bubonic plagues (Perry and Fetherston, 1997).

The events leading to the speciation of the *Yersiniae* genus have been reviewed in detail in (Wren, 2003). The ancestor of pathogenic *Yersiniae* derived from non-pathogenic environmental *Yersiniae* by acquisition of the plasmid pCD1 (Fig. 1). This plasmid contains genes encoding Type III secretion system (T3SS), which serve to subvert the activity of the immune system. The avoidance of the activity of the immune system had allowed the establishment of this clone into the new (infective) niche. Afterwards, acquisition of different elements allowed divergence of *Y. enterocolitica* and *Y. pseudotuberculosis*. The speciation of *Y. pestis* from *Y. pseudotuberculosis* is a recent process that has required not only gene acquisition, but gene loss as well. Two events are particularly important in such process. One is the rewiring of *Y. pestis* physiology due to the loss of genes encoding transcriptional regulators (Zhou and Yang, 2009). This change in the regulatory networks increases the adaptation of *Y. pestis* to its human host. A second event is the loss of genes encoding insect toxins. The loss of insect toxins,

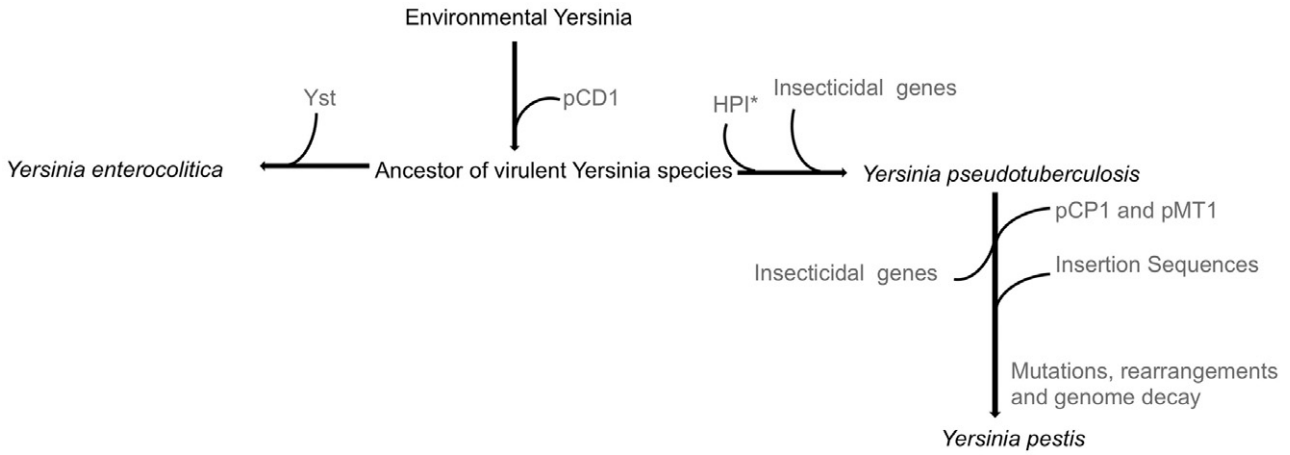

Fig. 1. Evolution of *Yersinia pestis*. The process of *Y. pestis* speciation from an environmental, non-pathogenic ancestor is a good example of the evolution steps that are involved in the emergence of bacterial pathogens. This process began with the acquisition of the plasmid pCD1 by environmental Yersinia. This plasmid harbours genes encoding virulence determinants as T3SS and effector Yop proteins. From this ancestor of virulent Yersinia species, two branches have evolved. One diverged through the acquisition of the Yersinia stable toxin (Yst) and had lead to the speciation of *Y. enterocolitica*. This species has further evolved through acquisition and loss of genes (not shown in this figure). The other branch diverged trough the acquisition of the high pathogenicity island (HPI*), which encodes an iron-uptake system and is present as well in different *Enterobacteriaceae*, and by the incorporation of insecticidal genes. *Yersinia pestis* is a successful clone that emerged recently from *Y. pseudotuberculosis* through the acquisition of the plasmids pCP1, which encodes the plasminogen activator gene and pMT1, which allows colonization of the gut of fleas. The loss of insect toxins is an important event for the persistence of *Y. pestis* in its insect vectors. The acquisition of insertion sequences is in the basis of the genome rearrangements and gene loss of *Y. pestis*. Finally, all the process of adaptation to a new host is modulated by the mutation-driven optimization of the regulatory and metabolic networks of the pathogen. This evolution process is described in more detail in Wren (2003), Keim and Wagner (2009) and Zhou and Yang (2009).

together with the increased capability of forming biofilms inside fleas are important elements in the evolution of *Y. pestis*, because they allow its transmission by bites of infected insects (Chouikha and Hinnebusch, 2012). We can speculate that one consequence of these adaptations is that *Y. pestis* is on the road towards commensalism in insects.

This example shows that the adaptation of a bacteria to colonize (infect) a new host requires the acquisition of novel elements by means of HGT (Fig. 2), eventually the loss of others that are non-adaptive in the novel environment and the rewiring of the bacterial physiology by means of mutations, mainly in regulatory elements (Zhou and Yang, 2009). A final step might be the loss of elements that are not needed in the novel infective environment (genome reduction). As a consequence, the new bacterial species may loss the capability of growing in the habitats of its parental, which will consequently speed up the process of speciation.

One aspect in this evolution process that has not been studied in detail is the effect of acquisition of genomic islands on the bacterial physiology. In the case of antibiotic resistance, it has been discussed that the acquisition of resistance genes can confer a competitive disadvantage (fitness cost) due to the metabolic load required for the replication, transcription and translation of the novel elements acquired by HGT. Recent works indicate that fitness costs can be more specific (Morosini *et al*., 2000; Sanchez *et al*., 2002; Alonso *et al*., 2004; Sanchez and Martinez, 2012) and that the acquisition of novel genes might alter specifically the metabolic bacterial networks (Martinez *et al*., 2011). In addition, the regulation of the expression of such elements requires to be integrated into the general bacterial regulatory networks (Linares *et al*., 2010; Martinez and Rojo, 2011).

Whereas antibiotic resistance evolution is recent and the ancestor and the evolved strains can be compared easily, the divergence of bacterial pathogens happened much earlier in the evolution and this type of experiments are more difficult to perform. However, the comparison of the regulatory networks of species from the same genus may provide valuable information on this issue. This exercise has been made for *Y. pestis* and *Y. pseudotuberculosis* (see Zhou and Yang, 2009 for more details). As discussed in the article, regulatory networks can change because of the loss of regulators or because of the loss of the targets of the regulators. These studies show the final consequences of acquiring novel virulence determinants for bacterial regulatory networks. However, by using this approach we cannot distinguish between the evolutionary constrains due to gene acquisition and those derived from the colonization (infection) of a new habitat. One alternative for analysing this issue is by studying how the introduction of new genomic islands challenges the bacterial transcriptome. The *Salmonella* genomic island 1 (SGI1) is

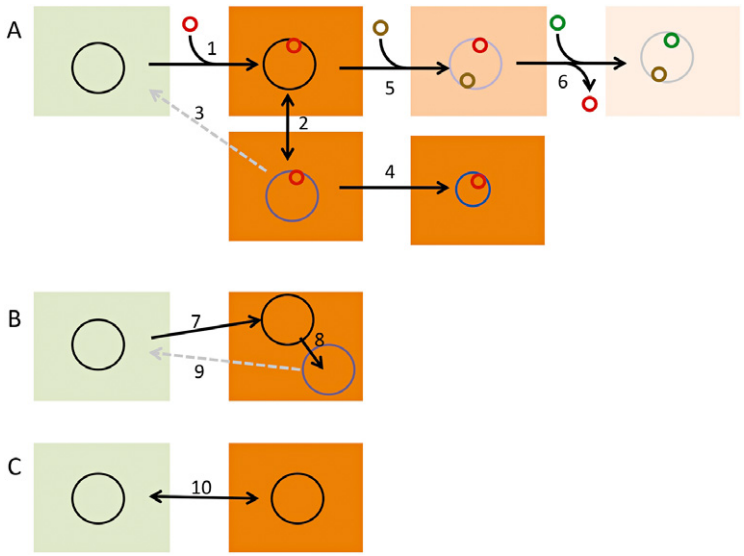

Fig. 2. Evolutionary trajectories of bacterial pathogens. Panel A shows the process of speciation of a pathogen (bigger circles in the figure) as *Y. pestis*. This process usually begins with the acquisition, by HGT, of a set of genes (red circle) that allow the shift of the pathogen's habitat from the environment to an infected host (1). If the rate of transmission is high enough, the newborn pathogen will disseminate among different individuals (2) and evolve by different mechanisms that include mutation and eventually genome reduction (4). These evolutionary processes might cause the de-adaptation of the pathogen to its original habitat in which case the chances of the microorganism to re-colonize natural ecosystems will be low (3). Once the organism is a pathogen, it can change host specificity by acquiring novel genes (5) and eventually the loss of determinants un-needed in the novel host (6). In all cases, the integration of the acquired elements into the preformed bacterial metabolic and regulatory networks will be tuned by mutation. Panel B shows the process of short-sighted evolution of opportunistic pathogens with an environmental origin like *P. aeruginosa*. These microorganisms infect patients presenting a basal disease, using virulence determinants already encoded in their genomes (7). During chronic infection, the infective strain evolves mainly by mutation and genome rearrangements (8). However, since it only infects people with a basal disease, transmission rates are usually low, which precludes clonal expansion and further diversification. Since adaptation to the new host is of no value for colonizing the environmental habitat (9), this is a dead-end evolution process. Panel C shows the evolution of pathogens as *V. cholerae* that present virulence determinants with a dual role in the environment and for infections, in which case the colonization of one of these two habitats does not severely compromise the colonization of the other (10).

an element that contains antibiotic resistance genes and other determinants that affect bacterial virulence, although the function of the latter has not been accurately determined. It has been shown that the expression of SGI1 alters the global gene expression of *Salmonella enterica* serovar Typhimurium (Golding *et al.*, 2007) in aspects relevant for the basic bacterial physiology that include iron utilization, chemotaxis and motility.

The study of opportunistic pathogens can shed light on the role of virulence factors in environmental (non-clinical) ecosystems

In several occasions, the time required to evolve a human pathogen is much larger than the time since humankind emerged as a new species. In other cases, the infective agent has evolved with humans ancestors (Trueba and Dunthorn, 2012) or the only modification required for the pathogen to infect humans has been the acquisition of elements allowing a host change. However, even for these 'recent' pathogens what is being measured is the time at which the acquisition of the virulence determinants has happened, not the time required for the evolution of these determinants themselves. Before their acquisition by bacterial pathogens, virulence determinants evolved in other hosts that are largely unknown, and their function in these hosts might be different than virulence. For understanding in more detail which are the functions that virulence determinants might have before their transfer to a pathogen, it would be useful to make a distinction between elements involved in the overall bacterial physiology and elements that are relevant for the bacterial/host interaction.

As stated above, pathogenicity islands may encode elements as iron-uptake systems or catabolic pathways (Schubert *et al.*, 1998; Hacker and Carniel, 2001; Luck *et al.*, 2001; Chouikha *et al.*, 2006). These elements are important for infecting a host, but are relevant as well in the field, where iron is scarcely available and there exist a variety of carbon sources. Virulence 'metabolic' elements, likely have a similar physiological role in their original hosts. In a similar way, virulence determinants as the capsule or the lipopolysaccharide, are elements of the bacterial structure and their role in virulence is just a consequence of the action of the host's mechanisms for detecting and eliminating pathogens.

A different situation concerns the determinants of virulence that are involved in the host/pathogen cross-talk. Examples of them are toxins or T3SS effectors that affect the host physiology. For these elements, a role on the basic metabolism of their original bacterial host cannot be foreseen. Rather, they should have originally evolved to play a role in the cross-talk between bacteria and eukaryotic cells in non-clinical ecosystems. The finding that environmental and clinical isolates of the free-living opportunistic pathogen *Pseudomonas aeruginosa* are genetically and functionally equivalent supports this statement (Alonso *et al*., 1999; Morales *et al*., 2004). *Pseudomonas aeruginosa* presents a T3SS (Engel and Balachandran, 2009), but in contrast to those present in classical pathogens, the genes encoding it are not located in a pathogenicity island. Furthermore, T3SS genes are distributed in different parts of the *P. aeruginosa* genome, indicating they are old elements in the evolution of this species, and not the result of a recent gene transfer event. In addition, *P. aeruginosa* produces toxic compounds as cyanide or pyocyanin that are relevant for its virulence (Goldfarb and Margraf, 1967; Liu, 1974; Gallagher and Manoil, 2001; Muller, 2002; O'Malley *et al*., 2003; Lau *et al*., 2004a,b; Allen *et al*., 2005; Broderick *et al*., 2008).

Quite importantly, it has been shown that the same virulence determinants required for infecting humans are also needed for infecting plants, protists, worms or insects (Rahme *et al*., 1995; 2000; Mahajan-Miklos *et al*., 1999; 2000; Cosson *et al*., 2002; Miyata *et al*., 2003; Carilla-Latorre *et al*., 2008). Taken into account the evolutionary tree and the different hosts infected by this bacterial species, it is worth thinking that some of these virulence determinants evolved before the emergence of multicellularity or mediate bacteria/plant interactions. The reasons for this evolution may lay on prey/predator and in commensal/host interactions. For instance, it has been shown that several plant-associated bacteria present T3SS that mediate bacteria/plant interactions (Hueck, 1998). Studies of experimental evolution have shown as well that protozoan grazing selects *Pseudomonas* mutants that overproduce the exopolysaccharide alginate (Matz *et al*., 2002). Alginate overproduction is frequent in *P. aeruginosa* cells causing chronic infections in cystic fibrosis patients (May *et al*., 1991). One of the consequences of this overproduction is that those cells cannot be engulfed by macrophages. Since this situation is the same observed when *P. aeruginosa* is confronted with a protozoan predator, we can imagine that this bacterial species has acquired this trait upon contact with protozoans in Nature (Matz *et al*., 2004; Weitere *et al*., 2005).

A similar situation has been described for the bacteria causing the Legionnaires' disease, *Legionella pneumophila.* This bacterial species is ubiquitously found in water, where it replicates within amoeba and ciliated protozoa. When infecting humans, *L. pneumophila* multiplies into alveolar macrophages. The relevant issue here is that the mechanisms for persisting inside macrophages are the same used by *L. pneumophila* for surviving inside its unicellular hosts (Gao *et al*., 1997).

Whether or not the virulence determinants currently present in pathogenicity islands had been originated in the environment either to colonize a host or to survive a predator remains to be fully established. However, recent works support this statement. For instance, it has been described that Shiga toxin serves bacteria to evade the predation by the bacteriovorus ciliate *Tetrahymena thermophila* (Steinberg and Levin, 2007; Lainhart *et al*., 2009). Similarly, the *Listeria monocytogenes* major virulence factor Listeriolysin O, which is required for intracellular survival of the pathogen during infection and for apoptosis induction in lymphocytes, allows bacterial survival from predation by *Tetrahymena pyriformis* (Pushkareva and Ermolaeva, 2010).

This dual role of virulence determinants for causing an infection and as fitness determinants in non-infective habitats has been described as well for other elements (Pruzzo *et al*., 2008; Vezzulli *et al*., 2008). This is the case for the colonization factor GbpA that mediates attachment of *V. cholerae* to epithelial cells and to chitin. This attachment enhances the capability of *V. cholerae* for colonizing the human gastrointestinal tract. It is important as well for the attachment of bacterium to the chitin-containing shells of small crustaceans present in the coastal water environments that are the natural habitats of *V. cholerae* (Kirn *et al*., 2005).

The evolution of virulence factors that interact with the host at the single cell level, can be driven by the interactions between bacteria and protists. However, the subversion of the immune system exerted by some bacterial pathogens requires a more complex host to evolve. It has been suggested that invertebrates, which have a well-developed immune system, might have shaped the evolution of some bacterial pathogens (Waterfield *et al*., 2004).

The increase of available genomic and metagenomic sequences may help to establish the original hosts of the virulence determinants currently present in human pathogens, as well as the putative non-pathogenic ancestors or such pathogens. Indeed, the sequence of deep-sea bacterial genomes has shown the existence of evolutionary links between pathogenic *Helicobacter* and *Campylobacter* species and their non-pathogenic, chemolithoautotrophic deep-sea relatives (Nakagawa *et al*., 2007).

Short-sighted evolution: when adaptation is not followed by speciation

Once a bacterium has acquired the capability to colonize (infect) a new host, it will evolve to further increase its

adaptation. This inside-host evolution will be mainly due to genomic changes that include mutations, gene loss and rearrangements, which altogether will render the emergence of a new species. However, even though the process of inside-host adaptation might occur for any type of infection, the consequences for bacterial evolution would not be always the same (Fig. 2). In occasions, as discussed above, the process ends up with the emergence of a new species. However, in other cases, the evolved organism is not being outcompeted once the infection finishes in a process that has been named as short-sighted evolution (Levin and Bull, 1994). This type of evolution may be relevant for chronic infection with low inter-patient transmission levels. This is the case of chronic infections produced by *P. aeruginosa* (Smith *et al*., 2006; Martinez-Solano *et al*., 2008; Mena *et al*., 2008). This bacterial species produces chronic infections in cystic fibrosis patients. Usually, each patient is colonized by a different clone, which evolves in the lungs of the person for decades. This mutant-driven evolution is frequently favoured by the selection of strains presenting high mutation rates (Oliver *et al*., 2000; Mena *et al*., 2008). Strains from different patients present similar evolutionary landscapes, a feature compatible with the fact that bacteria evolve to colonize habitats that are largely similar (the lung from each patient). However, once bacteria are released reinfection of another patient with the evolved bacterium is uncommon, and this clone is outcompeted in the natural habitat of *P. aeruginosa*. This situation can been discussed in the frame of a source-sink situation (Sokurenko *et al*., 2006). Natural ecosystems (source) contain a large number of bacteria only few of which infect some patients (sink) just by chance. Evolution during infection renders adaptation to the infected host and a concomitant de-adaptation to the original habitat. If inter-host transmission is high, this might allow further evolution and eventually speciation. However, if the fate of the evolved strain is to return to its source, it will be outcompeted and will disappear (Fig. 2). If the rate of inter-host transmission is a relevant issue for the process, speciation might be favoured for epidemic clones, a feature that might be currently happening in the case of *P. aeruginosa* epidemic strains isolated in Denmark (Jelsbak *et al*., 2007; Rau *et al*., 2010; Yang *et al*., 2011; Hansen *et al*., 2012).

Ecology of *V. cholerae*: a phage tale

Vibrio cholerae is a water-borne bacterial pathogen that cause epidemics in different parts of the world (Charles and Ryan, 2011). Infection is achieved by drinking water contaminated by virulent strains of this bacterial species. Besides being a relevant pathogen, *V. cholerae* is a part of the free-living marine coastal microbiota, which suggests that the behaviour of this bacterial species in natural (non-clinical) ecosystems might be of relevance for cholera outbreaks, mainly in endemic areas. In fact, the seasonal raising of outbreaks at endemic areas has been attributed to changes in the temperature of surface waters. Increased temperature is associated with plankton blooms and increased amount of *V. cholerae* in water and, as a consequence, increased chances for ingesting the infectious dose for developing cholerae (Lipp *et al*., 2002). However, the ecology of *V. cholerae* is not that simple and its capability for producing epidemics relies on the presence of bacteriophages in natural ecosystems (Faruque *et al*., 1998; Jensen *et al*., 2006). The main virulence factor of *V. cholerae* is a toxin that is encoded by the lysogenic phage CTXφ. The prevalence of toxigenic strains containing this phage in water ecosystems is low. However, the release of stools of infected patients containing toxigenic *V. cholerae* increases, not just the number of these strains, but also the probability of spread of CTXφ. Furthermore, the phage can be integrated as well in other non-pathogenic *Vibrio* as *V. mimicus* that can act as reservoirs and as enhancers for the conversion of non-toxigenic towards toxigenic variants of *V. cholerae*. Whereas lysogenic phages increase the population of virulent *V. cholerae*, lythic phages reduce such population (Faruque *et al*., 2005). Indeed, it has been discussed that the decay in water of *V. cholerae* outbursts is modulated by trophic relationships that include predation by bacteriovorus protozoans and the action of bacteriophages. The conclusion from these studies is that the epidemics of cholera are modulated by the behaviour of *V. cholerae* in its natural habitat, coastal waters (Worden *et al*., 2006). Changes in the temperature of the water that may be local or associated to global warming might alter the composition of the water microbiota in general and the abundance of *V. cholerae* in particular. This observation highlights the relevant role that natural ecosystems have on the emergence and spread of pathogens.

Concluding remarks

The research on virulent bacteria relies mainly on the studies on the interaction of the pathogen with its human host or with a surrogate model. However, increased knowledge in the field targets natural, non-human reservoirs as the origin of virulence determinants. In occasions those determinants have been acquired from an unknown host and are useful just for the colonization of the infected host. In other cases they serve for the adaptation of current bacterial pathogens to different lifestyles, which include both infective and non-infective environments. The fact that simple bacteriovorous models like *Caenorhabditis elegans*, *Dictyostelium discoideum* or *Tetrahymena* are useful for studying the virulence of several

pathogens indicates that virulence determinants can be of relevance for the trophic chain and in general for prey/predator relationships. In occasions, as it happens with iron-uptake systems or proteases, a direct role of the virulence determinant for nutrient acquisition can be foreseen. In other cases, as T3SS, the production of toxins or the synthesis of colonization factors, the impact will be on prey/predator (or commensal) relationships. For those pathogens that have environmental reservoirs, the study of these trophic relationships might be relevant for a full understanding of the emergence and spread of infections in humans.

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