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Microreview

Molecular mechanisms underlying the emergence of bacterial pathogens: an ecological perspective

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SUMMARY

The rapid emergence of new bacterial diseases negatively affects both human health and agricultural productivity. Although the molecular mechanisms underlying these disease emergences are shared between human- and plant-pathogenic bacteria, not much effort has been made to date to understand disease emergences caused by plant-pathogenic bacteria. In particular, there is a paucity of information in the literature on the role of environmental habitats in which plant-pathogenic bacteria evolve and on the stress factors to which these microbes are unceasingly exposed. In this microreview, we focus on three molecular mechanisms underlying pathogenicity in bacteria, namely mutations, genomic rearrangements and the acquisition of new DNA sequences through horizontal gene transfer (HGT). We briefly discuss the role of these mechanisms in bacterial disease emergence and elucidate how the environment can influence the occurrence and regulation of these molecular mechanisms by directly impacting disease emergence. The understanding of such molecular evolutionary mechanisms and their environmental drivers will represent an important step towards predicting bacterial disease emergence and developing sustainable management strategies for crops.

Keywords: disease emergence, environmental habitats, genomic rearrangements, horizontal gene transfer, point mutations.

INTRODUCTION

Emerging diseases can be defined as infections that have either newly appeared in a given population or have historically existed and are once more spreading in incidence or geographical range (Morse, 1995). In the last 40 years, 335 new infectious diseases have emerged in humans, the majority of which are caused by multidrug-resistant bacteria (Jones *et al*., 2008). Likewise, the number of plant disease outbreaks, some caused by plant-pathogenic bacteria, has also increased in recent decades. For example, since the beginning of this century, the phytopathogen *Pseudomonas syringae* has caused over 55 disease outbreaks in perennial plants (Lamichhane *et al*., 2014) and over 70 disease outbreaks in annual plants (Lamichhane *et al*., 2015). Such an increasing number of disease outbreaks raises concerns about the origin of the genetic lines causing such epidemics, as well as the evolutionary processes involved.

A large number of infectious diseases are caused by host-adapted microbial agents which evolve a pathogenic phenotype through the acquisition of new pathogenic determinants (Morens and Fauci, 2013). In addition, most of the disease outbreaks are caused by different bacterial genetic lineages. The understanding of the molecular mechanisms underlying the emergence of such bacterial genetic lines therefore appears to be a fundamental step towards predicting disease emergence.

Overall, three mechanisms are behind the origin of DNA modifications that lead to the evolution of genomic traits underlying pathogenicity in bacteria: (i) point mutations (small local sequence change); (ii) rearrangements of DNA segments (such as gene duplications or insertion/deletion of entire or portions of genes); and (iii) the acquisition of new DNA components from other organisms via horizontal gene transfer (HGT) (Arber, 2008). The result of these processes is an altered bacterial phenotype which could be either beneficial or harmful for bacteria depending on the environment they encounter. In addition, whether or not the new alleles coming from genomic modifications will be fixed into a given bacterial population is strictly related to the effect of genetic drift and natural selection, both of which are strongly influenced by environmental fluctuations (Ohta, 2000).

However, the emergence of new diseases is also affected by a combination of ecological, environmental and socio-economic factors (Morens *et al*., 2004). Most of the emerging diseases seem to be caused by existing pathogens which, through adaptation to new habitats, evolve the capability to cause disease in

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new hosts. For instance, zoonoses, phytonoses and sapronoses are examples of how the reservoirs of the aetiological agents represent habitats in which such agents survive and replicate (Hubálek, 2002; Morens and Fauci, 2013; van Overbeek *et al*., 2014). Overall, such agents could be non-pathogenic or less aggressive to their co-evolved hosts when compared with newly infected hosts (in the case of phytonoses and zoonoses). In terms of zoonoses, a recent World Health Organization report has identified over 200 diseases, including those of bacterial and viral origin. There is evidence that the *Ebola virus* first spread as a single zoonotic transmission, followed by a subsequent human–human transmission (Gire *et al*., 2014). A recent study has suggested that bats may act as a reservoir of *Ebola virus* (Saez *et al*., 2014). Like viruses, bacterial pathogens may originate from human-altered environments which act as reservoirs of pathogenic strains (Morris *et al*., 2009). A report from the World Health Organization includes the shiga toxin-producing *Escherichia coli* O157:H7 and *Salmonella enterica* among the most virulent food-borne bacterial pathogens. Both of these bacterial species gained access to the food chain as a result of poor hygiene practices (Morens *et al*., 2004). There are also reports of zoonotic agents coming from different reservoirs: *Francisella tularensis*, the causative agent of tularaemia disease, was found in over 100 vertebrate species and, to date, no specific reservoir of this bacterium has been identified (Mailles and Vaillant, 2014). Like for human pathogens, both abiotic and biotic environments can act as potential reservoirs of plant-pathogenic bacteria. These bacterial lineages can evolve under pressures that are different from those exerted by the final host plants. Recent studies have emphasized the role of water habitats in the evolution of *Pseudomonas syringae* lineages pathogenic to kiwifruit and tomato plants (Bartoli *et al*., 2015a; Monteil *et al*., 2013). In addition, plants are likely to play an important role in disease emergence by acting as reservoirs of pathogenic lineages, as, for example, observed in *Xanthomonas campestris* strains (Toussaint *et al*., 2012). More generally, under the selective pressure of resistant and/or tolerant plants, bacteria can evolve new allelic forms of virulence which, in turn, may trigger disease on new hosts. In particular, tolerant plants seem to be much more suitable for bacterial evolution, as tolerance is attained by limiting pathogen damage without reducing bacterial growth (i.e. without reducing population size) (J. R. Lamichhane *et al*., unpublished data). Here, we summarize the role of point mutations, genomic rearrangements and HGT in the emergence of bacterial lineages pathogenic to either plants or humans, with particular emphasis on the role of the environment in triggering these mechanisms. The understanding of the molecular evolutionary mechanisms underlying bacterial disease emergence and the role of the environment in this evolutionary scenario could shed light on the possible development of sustainable disease management strategies.

MUTATIONS AND PATHOADAPTIVE MUTATIONS ARE THE SMALLEST GENOMIC CHANGES WHICH LEAD TO RAPID EVOLUTION OF BACTERIAL LINEAGES IN A FLUCTUATING ENVIRONMENT

Mutation is one of the major sources of DNA modification underlying the adaptation of bacteria to new environments. Although mutations can result from encounters with chemical mutagenic agents (Arber, 2008), they generally occur as the consequence of replication machinery mistakes. Prokaryotes, like eukaryotes, repair their post-replicative mistakes via the methyl-directed mismatch repair (MMR) system (Schofield and Hsieh, 2003). When the MMR system is defective, mutations are incorporated into the DNA of the replicating cell, a process related to the occurrence of variability.

Mutations can either directly confer a newly pathogenic phenotype or enhance its aggressiveness. These mutations are the product of an evolutionary process called pathoadaptive mutation which confers or enhances bacterial pathogenicity without HGT (Sokurenko *et al*., 1999). Although pathoadaptive mutations in human pathogens have been studied extensively, there is a paucity of information regarding these mutations in plant-pathogenic bacteria. In *Pseudomonas aeruginosa*, knockout mutations in *mucA*, a repressor of alginate biosynthesis, lead to the over-expression of the *algU* gene involved in alginate biosynthesis (important for pulmonary tract colonization), thereby promoting the colonization of the lung by the pathogen (Boucher *et al*., 1997). In *Yersinia pestis*, mutations on *yopA*, involved in neutrophil adhesion, enhance the ability of the bacterium to colonize the host by evading phagocytes (Rosqvist *et al*., 1988). In *P. syringae* strains, the *hopZ1* effector gene, known to promote infection in soybean (Zhou *et al*., 2011), has evolved into three functional and two non-functional forms (Ma *et al*., 2006) as a consequence of bacterial–host interactions in which the ancient *hopZ1* evolved towards a more effective one (Ma *et al*., 2006). Likewise, in *Xanthomonas axonopodis* pv. *vesicatoria*, point mutations in *avrBs2* enable evasion of Bs2 recognition for some strains isolated from peppers in the field (Wichmann *et al*., 2005).

Experimental evolution is a powerful tool to investigate pathogen evolution, as well as to follow up the occurrence of mutations related to host adaptation and pathogenicity (Bataillon *et al*., 2013). A recent evolutionary study on *Ralstonia solanacearum* (Guidot *et al*., 2014) demonstrated that, although a recurrent inoculation passage of the bacterium on its original host (i.e. tomato plant in which the bacterium is reported to cause disease symptoms) leads to an enhancement of *R. solanacearum* fitness on tomato, a similar passage on a distant host (i.e. bean plant in which the bacterium replicates without inducing disease symptoms) leads to an adaptation to bean plants. The adaptation to a

distant host was caused by mutations in the regulatory *RSc1097* gene that *R. solanacearum* evolved during plant infection (Guidot *et al*., 2014). Likewise, several inoculation passages of *X. citri* ssp. *citri* on a resistant Meiwa kumquat line have led to the selection of bacterial strains that are no longer able to induce a highly local resistance response—which was often associated with the hypersensitive response (HR)—in the host plant (Trivedi and Wang, 2014). The loss of HR—a process used by *X. citri* strains to evade plant immune defences—was associated with non-synonymous mutations in the effector genes *avrXacE1*, *pthA2* and *avrXacA3*; such effectors are known to elicit HR on resistant hosts (Trivedi and Wang, 2014). The experimental evolution studies mentioned above identified the pathogens' pathoadaptive mutations in controlled conditions in which their selection was the result of recurrent passages on the host plants. However, bacterial pathogens might have different phases outside their host (i.e. in environmental habitats that are different from plants), where other factors could affect the evolutionary dynamics of pathogenic determinants. For example, less aggressive strains of *P. syringae* have been demonstrated to have a fitness advantage in the presence of virulent conspecifics *in planta*, whereas there was a fitness disadvantage in soil conditions (Barrett *et al*., 2011). Recent studies have also highlighted a possible scenario in which *P. syringae* strains inhabiting water habitats could rapidly evolve under environmental conditions that differ from their final host plants (Bartoli *et al*., 2015a; Monteil *et al*., 2013). However, none of these studies has demonstrated the capacity of *P. syringae* to experience evolution trajectories in non-crop habitats with consequences on the pathogenicity of this bacterium on crops.

Hypermutable bacterial cells (cells showing a higher mutation rate compared with their wild-type counterparts) constitute a particular case in which mutations rapidly accumulate in a given bacterial cell by strongly influencing the bacterial phenotype, including pathogenicity. Overall, mutators experience accelerated adaptive evolution which can be linked to the adaptation to new environments (Remigi *et al*., 2014). If mutations reside in genes that enable bacteria to infect new hosts, a bacterial mutator population can rapidly emerge causing disease emergences. For example, the mutation rate of *Helicobacter pylori*, the causal agent of acute ulcers, increases during the acute infection phase. This mutation burst decreases during chronic infection, suggesting that an elevated mutation rate helps *H. pylori* to adapt to and colonize human stomach (Linz *et al*., 2014). Hypermutators often exhibit a defective MMR system that leads directly to antibiotic resistance (Meyers and Bull, 2002; Richardson *et al*., 2002), a process that has a serious impact on human health and disease control. However, recent studies on plant-pathogenic *Pseudomonas viridiflava* have demonstrated that, although mutability is positively associated with the occurrence of antibiotic-resistant bacterial lines, it is negatively associated with pathogenicity (Bartoli *et al*., 2015b). This finding raises questions about whether or not hypermutable antibiotic-resistant cells maintain their aggressiveness in human bacterial pathogens.

However, the environment can directly influence the occurrence of mutations. For instance, reactive oxygen species (ROS)—which are generated in the environment by different processes (Gracy *et al*., 1999)—can interfere with MMR gene expression, leading to hypermutable phenotype conversion in *P. aeruginosa* (Torres-Barceló *et al*., 2013). During plant infections, ROS produced by plants could directly inhibit the MMR system of the colonizing bacterium with a consequence on its mutation rate. Particular environmental conditions, such as starvation, can induce mutagenesis leading to antibiotic resistance (Poole, 2012). Taken together, particular environmental stresses could enhance the occurrence of mutations, leading to a better adaptation of bacteria to fluctuating environments. For this reason, we propose that experimental evolution studies that focus on a better understanding of the evolutionary trajectories of pathogenic determinants, via pathoadaptive mutation processes, should be performed in conditions that are close to the natural conditions to which both pathogens and hosts are continuously exposed.

The approaches used in a given evolutionary experiment could dramatically influence the outcome of the analysis with a consequent misinterpretation of the prediction of the evolution of a given organism in natural conditions. Two parallel studies on the avian H5N1 influenza A virus—both aiming to understand whether the virus could acquire mutations conferring the ability to be transmitted in mammals—led to different biological conclusions. In the first study, hybrid virus was first created and random mutations were introduced in a viral region containing a haemagglutinin protein which is known to determine host range specificity (Imai *et al*., 2012). The authors demonstrated that four mutations on the hybrid virus were needed to enable its transmission from ferret to ferret and that the resulting virus maintained its aggressiveness. By contrast, in the second study, the wild-type H5N1 virus was allowed to randomly evolve after artificial infection passages in ferrets (Herfst *et al*., 2012). The authors showed that five mutations were needed to enable H5N1 to be transmitted from ferret to ferret, although the transmissible virus was much less aggressive in ferrets and was more susceptible to antiviral drugs. In plant pathology, experimental evolution studies can be more easily performed on the 'natural' hosts of the pathogens. However, serial passages are likely to be far from representative of natural infections when performed in controlled conditions. For example, a given bacterial pathogen evolves in plants with the whole microbiota, which may differ markedly between controlled and field conditions. All of these observations suggest that experimental evolution studies, in monitoring pathogen evolution in plants, may provide more reliable predictions of the evolutionary trajectories of the pathogens if such studies are conducted in ecologically realistic conditions.

GENOMIC REARRANGEMENTS: THE ENHANCERS OF PATHOGENICITY UNDER STRESSFUL ENVIRONMENTAL CONDITIONS?

Genomic rearrangements (deletions, inversions and duplications) are molecular mechanisms that occur through the recombination between homologous sequences, such as transposons, mobile genetic elements, insertion sequence (IS) elements and prophages (Anderson and Roth, 1981; Cui *et al*., 2012; Iguchi *et al*., 2006). This mechanism has significant effects on bacterial phenotypes, including traits associated with pathogenicity and virulence (Jackson *et al*., 2011). Genomic rearrangements can profoundly affect gene expression, leading to the complete loss of gene function when the rearrangement falls in reading frames (Darling *et al*., 2008).

Several human and plant bacterial pathogens exhibit a reduced genome size compared with other non-pathogenic bacteria because of frequent genomic rearrangements (Jackson *et al*., 2011). These genomic reductions in specialized pathogens are often associated with host adaptation. A long-term study on patients affected by cystic fibrosis showed that 8% of the *P. aeruginosa* genome was deleted following an adaptation to the human environment (12-fold greater than in strains that evolved *in vitro*; Rau *et al*., 2012). Evidence that genomic rearrangements lead to the acquisition of pathogenicity traits also exists in phytopathogenic bacteria. *In planta* experiments have demonstrated that, during bean infection, *P. syringae* pv. *phaseolicola* loses the conjugative PPHGI-1 element, carrier of the avirulent gene *hopAR1* which triggers immune responses in bean plants. The loss of PPHGI-1 leads to disease development in the host plant (Lovell *et al*., 2009). In *P. syringae*, the exchangeable effector locus (EEL), which is part of the type III secretion system (T3SS) of this bacterium and harbours effector genes important for both pathogenicity and host range, contains mobile and IS elements (Alfano *et al*., 2000). The presence of these mobile elements suggests that EEL is often the target of recurrent genomic rearrangements, potentially facilitating host adaptation, with direct consequences on the emergence of pathogenic *P. syringae* lineages.

The relationship between environmental stresses and the occurrence of rearrangements in genomic regions with IS has been demonstrated in only a few cases. In a glucose-limited environment, inversions, deletions and duplications located close to IS elements, have been shown to confer a rapid fitness enhancement in 12 evolved *E. coli* strains (Raeside *et al*., 2014). Deletion and duplication events have also been observed in *E. coli* strains under high-temperature conditions as a response to thermal adaptation (Riehle *et al*., 2001). The latter study suggests that, in the context of ongoing climate change, bacteria might be more likely to rearrange their genome to rapidly adapt to fluctuating temperatures. Genomic rearrangements have also been shown to play an important role in the adaptation of *Thermus* species to human-made pullulate environments (Kumwenda *et al*., 2014). The nature of other environmental factors driving genomic rearrangements and the molecular mechanisms that influence such rearrangements still remain open questions, particularly in the case of pathogenicity acquisition.

The first step in understanding the factors driving genomic rearrangements requires a better identification of such rearrangements. To date, the detection of genomic rearrangements is still limited because of the short reads produced by the current next-generation sequencing technologies. Consequently, assembling methods on next-generation sequence data often fail to assemble regions containing IS or other transposable sequences associated with genomic rearrangements. With the ever more successful next-generation sequencing technologies that allow the sequencing of >1-kb reads (e.g. Pacific Biosciences Menlo Park, CA, USA), the investigation and detection of genomic rearrangements in evolving bacterial populations will be facilitated (Larsen *et al*., 2014).

HGT: THE DIRECT ACQUISITION OF NEW PATHOGENIC DETERMINANTS OCCURRING INSIDE AND OUTSIDE THE HOST

HGT consists of the movement of genetic material from a 'donor' to a 'recipient' cell, which can lead to either modest or profound genomic differences among closely related bacterial strains (Polz *et al*., 2013). HGT, one of the powerful evolutionary processes at the molecular level, can immediately change the phenotype of bacteria, including their ability to colonize new habitats, differentiate in new niches and infect new hosts. Several studies have demonstrated that HGT is structured by habitats and ecology, rather than by geography and phylogeny, i.e. donors and recipients usually reside in the same habitat and share the same 'ecological behaviour' (Boucher *et al*., 1997; Polz *et al*., 2013; Popa *et al*., 2011). In particular, genes acquired horizontally form networks among bacteria that reflect the species niche specialization of these bacteria (Popa *et al*., 2011). For example, a recent study from the human microbioma demonstrated that the human body is shaped by different niches in which bacteria are clustered (Smillie *et al*., 2011). Within these niches, bacteria experience high levels of HGT independently from their phylogenetic histories. This process facilitates the colonization of a given niche by new bacterial species (Smillie *et al*., 2011).

Several studies on human bacterial pathogens have demonstrated how a bacterial lineage can rapidly shift from a weakly to a highly aggressive form through the acquisition of virulence determinants via HGT processes. Pathogenic lineages of *Staphylococcus aureus* evolved from several HGT events, leading to the acquisition of plasmids, pathogenicity islands (carrying genes important for pathogenicity of bacteria), bacteriophage and other transferred genomic determinants (Lindsay, 2010). A recent study

has shown that the acquisition of an arginine catabolic mobile element harbouring spermidine genes from *Staphylococcus epidermidis* provided an advantage to *S. aureus* during skin colonization (Planet *et al*., 2013).

Non-host-adapted bacterial lineages can evolve into host-specialized pathogenic lineages via HGT. An example is the *Yersinia* genus in which non-pathogenic lineages horizontally acquired the pCD1 plasmid carrying T3SS that led to the evolution of the primordial non-pathogenic *Yersinia* into an aggressive human pathogen (Wren, 2003; Zhou *et al*., 2004).

HGT also has an undisputed role in the emergence of phytopathogenic bacteria. For example, the presence of IS elements, integrases, transposases and different G + C contents suggests that pathogenicity islands spread among bacteria via HGT processes, thereby enabling them to invade their hosts (Hacker *et al*., 1997). Most plant-pathogenic bacteria—*Pseudomonas*, *Erwinia*, *Xanthomonas* and *Ralstonia*—have evolved two different *hrp/hrc* cluster groups that encode for the central core of T3SS. The discrepancy between the presence/absence of these two cluster groups and the phylogeny of different bacterial species has provided strong evidence that the *hrp/hrc* cluster has been horizontally acquired (Alfano and Collmer, 1997; Araki *et al*., 2006). The acquisition of a plasmid harbouring a *hrp/hrc* gene cluster, T3SS effectors and a gene cluster encoding for the biosynthesis of indole-3-acetic acid transformed the epiphytic *Pantoa agglomerans* into a host-specific, gall-forming pathogen of two different host plants, i.e. gypsophila and sugar beet (Barash and Manulis-Sasson, 2009). *In silico* whole-genome analysis constitutes a powerful tool to investigate genomic regions that are under HGT and to understand whether these regions could affect the pathogenic behaviour of a given bacterial species. For example, a study on the whole genome of *X. campestris* pv. *campestris* and *X. axonopodis* pv. *citri* revealed that genes associated with pathogenicity and suppression of host immune defences have been acquired by HGT during the evolutionary histories of these phytopathogenic bacteria (Lima *et al*., 2008).

The bacterial genome can also show traces of prophages—i.e. bacteriophages integrated into the host bacterial genome, following infection. These integrative elements can be considered as a particular case of HGT. In addition, prophages can easily be transferred horizontally among bacteria as they are often associated with gene transfer agents (Varani *et al*., 2013). Prophage, once integrated into the genome, confers novel phenotypic properties to the recipient bacterial cell, including the pathogenicity trait (Canchaya *et al*., 2004). The relationship between prophages and disease emergence has been studied intensively in the medical field, such as the prophage of *Salmonella typhimurium* carrying the SopE effector protein (Mirold *et al*., 1999) and the *Shigella flexneri* prophage Sf6 harbouring genes that encode for the lipopolysaccharide (LPS) virulence factors (Clark *et al*., 1991). By contrast, the direct link between phages and pathogenicity traits has been much less well investigated in plant-pathogenic bacteria. There is evidence that effector proteins and other virulent genes are flanked by phage DNA sequences (Varani *et al*., 2013), suggesting that phages play an important role in the pathogenicity of phytopathogenic bacteria. Studies on the two prophages SC1 and SC2 of *Candidatus Liberibacter asiaticus*, the causal agent of citrus greening, showed that their activation may be regulated by ROS stress produced during infection in non-host plants (Zhang *et al*., 2011). In particular, the expression of the lytic phase induced by the bacterium was higher during non-host plant infection (i.e. periwinkle), thereby suggesting a lack of adaptation of *Ca. L. asiaticus* to periwinkle cells. By contrast, no lytic phase was induced on citrus, the natural host of *Ca. L. asiaticus*. In other words, induction of the SC1 prophage by the bacterium was shown to be responsible for the host specificity of *Ca. L. asiaticus* (Fleites *et al*., 2014).

Particular environmental conditions markedly influence bacterial competence, which is the first step in gene uptake. In general, stressful environmental conditions alter bacterial competency to uptake DNA, thereby facilitating bacteria to acquire genomic determinants that might enhance their fitness in hostile environments (Jackson *et al*., 2011). For example, in *Pseudomonas stutzeri*, the activation of genes for cell competence, and consequently HGT, occurs only under low nutritional environments as an adaptive strategy based on the acquisition of genomic determinants triggered by poor food supplies (Bertolla and Simonet, 1999). Likewise, extreme environmental conditions, such as high salinity or temperature, can influence cell competence with an effect on HGT occurrence. Bacteria from high-salinity waters have been shown to express gene transfer agents (proteins that aid HGT in bacteria; McDaniel *et al*., 2012), suggesting that such environments can represent reservoirs of bacteria characterized by a high ability to acquire new genomic elements with putative consequences on disease emergence. Interestingly, in contrast with stressful conditions, particular high nutritional levels that allow the maintenance of high bacterial concentrations have been demonstrated to be essential for HGT occurrence (Dröge *et al*., 1998). Therefore, one can speculate that plants are optimal environments in which HGT might occur among bacterial species. Several studies have also identified water habitats as reservoirs of HGT processes for bacteria (reviewed by Lupo *et al*., 2012). In water habitats, favourable conditions for HGT are shaped by filter-feeding organisms or a biofilm matrix, both of which concentrate numerous bacterial species at high densities, thereby enhancing HGT processes (Molin and Tolker-Nielsen, 2003). Because phages are much more abundant than bacterial cells in water habitats (Srinivasiah *et al*., 2008), it can be deduced that such habitats can play an important role in HGT (from phages to bacteria) of pathogenic determinants (Colomer-Lluch *et al*., 2011).

Ecological studies that aim to understand where HGT processes occur and under what conditions—such as whole-genome comparisons of different pathogenic and non-pathogenic bacterial lineages—in combination with experimental evolution approaches applied to these bacterial lineages, could help us understand how a non-pathogenic strain can rapidly evolve, adapt to hosts and spread, thereby causing disease epidemics. The ecological data on the distribution of *P. syringae* in non-agricultural habitats showed the importance of looking beyond the host (Bartoli *et al*., 2015a; Morris *et al*., 2007, 2010). Although comparative genomics studies have been performed on non-crop and weakly pathogenic *P. syringae* strains, evolution experiments that aim to demonstrate whether these strains might evolve into aggressive pathogens via HGT or other molecular evolutionary mechanisms are still lacking.

CONCLUSIONS

Infectious bacterial diseases emerge as a consequence of molecular evolutionary processes underlying DNA modifications naturally experienced by bacteria. The mechanisms underlying these molecular evolutionary processes can be affected by several environmental factors to which bacteria are exposed. As a consequence, the rearrangements of pre-existing pathogenic determinants or the acquisition of new ones can occur in environmental conditions completely different from those of the final host plants. In order to better understand disease emergences and to develop sustainable management practices, we strongly encourage interdisciplinary studies that merge the fields of ecology, pathology, genomics and molecular biology. In particular, we suggest setting up: (i) parallel experiments in both controlled and field conditions to investigate whether DNA modifications are driven by environmental factors that are different from those in host plants; (ii) experiments that simulate stressful conditions to understand whether these conditions are the major forces regulating cell competence and mutations; and (iii) field experiments that aim to study disease emergence in the context of plant communities that might be reservoirs of pathogenic bacteria.

With the continuing development of next-generation sequencing technologies, population genomics has become a low-cost tool to study the epidemiology, aetiology and evolution of bacteria causing emerging diseases. In human bacterial pathogens, several studies have demonstrated the power of population genomics in understanding the virulence factors determining pathogen spread (reviewed by Wilson, 2012). The sampling strategies adopted to investigate the evolution of pathogens under a population approach can strongly influence the biological conclusions of a given study (Vinatzer and Monteil, 2014). Unlike human bacterial pathogens—where some studies on pathogenic strains have been performed at the population level—most evolutionary studies in plant pathology have lacked a population sampling approach. Recently, Karasov *et al*. (2014) have adopted a population approach whilst studying *Arabidopsis thaliana* and its co-inhabiting *P. syringae* populations, and have revealed new insights into the evolutionary trajectories of the plant resistant *R* gene *RPS5* and the corresponding bacterial effector *avrPphB*. Based on their results, the authors concluded that a long-lived polymorphism in the gene RPS5 was more likely to be maintained through complex and diffuse community-wide interactions and probably not through a tightly coupled interaction involving a single co-evolved *R* gene and effector pair (Karasov *et al*., 2014). Therefore, we urge future studies that aim to understand the evolution of plant-pathogenic bacteria in a context of disease emergence to carefully consider population samples.

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