

Highlight

Horizontal gene transfer gone wild: promiscuity in a kiwifruit pathogen leads to resistance to chemical control

Steven E. Lindow*

Department of Plant and Microbial Biology, University of California, Berkeley, CA, 94720, USA

Unlike most crop plants which have been domesticated over a period of hundreds if not thousands of years, kiwifruit (*Actinidia* spp.) has been grown commercially and at large scales only for about 50 years (Bartoli *et al.*, 2015). For that reason, it has been much more feasible to understand the origin of plant diseases on such a crop since its disease history has been well documented and collections have been made of its pathogens, enabling them to be interrogated using powerful new molecular approaches. During its short history of cultivation, at least four apparently independent incidences of the emergence of diseases caused by the bacterium *Pseudomonas syringae* have been noted on kiwifruit at various locations around the world (Bartoli *et al.*, 2015; McCann *et al.*, 2013). Most recently, a particularly devastating epidemic caused by a strain of *P. syringae* pv. *actinidiae* (*Psa*) that is quite distinct from those causing previous outbreaks of so-called canker diseases of kiwifruit appeared in Italy in 2008 and has subsequently spread to most other kiwifruit growing areas around the world (McCann *et al.*, 2013). *Pseudomonas syringae* is a very diverse, cosmopolitan bacterium that is commonly found as an epiphyte on healthy plants, in other natural reservoirs such as freshwaters, is readily disseminated globally via airborne particles from which it can be deposited via nucleation of supercooled water drops in clouds, as well as a pathogen of a variety of plant species (Morris *et al.*, 2008). Such a large potential reservoir of diverse *P. syringae* strains, even in nonagricultural environments, is likely the fodder by which a variety of newly

important variants that have proven to be highly pathogenic to important crop plants have been derived (Bartoli *et al.*, 2015; McCann *et al.*, 2013). Genome sequence-driven population genetic analyses of *Psa* strains support a model whereby recombination and horizontal gene transfer from *P. syringae* strains associated with wild *Actinidia* species, and likely those from other plant species as well, has led to the current epidemic *Psa* strain that is now devastating cultivated kiwifruit worldwide (McCann *et al.*, 2013).

The control of bacterial diseases of plants is particularly problematic as there are a limited number of bactericidal or bacteriostatic compounds available for this use. Furthermore, the spatial structuring of plant pathogens in or on plants make it difficult for topically applied bactericides to access pathogens, particularly when they are within plants. Although a few antibiotics, notably streptomycin and oxytetracycline are successfully used for disease control on a few crop plants, their use is prohibited in many parts of the world, and resistance to these antibiotics has frequently occurred in target pathogen populations, thereby limiting their use. The most commonly used bactericides for plant disease control are various copper compounds, notably the relatively insoluble cupric hydroxide formulations which are topically applied to plants, providing a source of copper ions that are slowly released, killing at least some of the susceptible bacterial cells in or on the plants. While not highly efficacious, such copper compounds are the sole means of chemical control of bacterial canker of kiwifruit caused by *Psa*. In an alarming finding reported in a recent issue of environmental microbiology, Colombi *et al.* (2017) describe how the epidemic *Psa* strain circulating in New Zealand since 2010 was able to acquire genes conferring resistance to copper bactericides within 4 years of its introduction. Detailed molecular investigation on several strains isolated after its first appearance, however, has provided a fascinating story of the apparent promiscuity of *Psa* and the myriad of different ways in which copper

*For correspondence. E-mail icelab@berkeley.edu; Tel. (1) 510-642-4174; Fax (1) 510-642-4995.

resistance has evolved in this pathogen, occurring from horizontal gene transfer mediated by acquisition of both plasmids and integrative conjugative elements (ICEs). While the plasmid-borne copper resistance determinants conferred somewhat higher levels of tolerance of copper ions than did those from ICEs, both have enabled *Psa* to escape control from copper bactericides, and both type of events represent strong evidence for incredibly high rates of horizontal transfer in the natural environment.

ICEs are entities with features like plasmids but also that of temperate phage. As such they can disseminate vertically as part of the bacterial core chromosome but also are capable of extensive horizontal movement via conjugation (Wozniak and Waldor, 2010). ICEs, such as those found in *Psa* are often quite large (> 100 kb), carrying genes of adaptive significance to their hosts. The most recent epidemic strain of *Psa* harbored one such ICE, (harboring an enolase, termed ICE_eno) integrated into the particular att-1 site in the chromosome. Fascinatingly however, investigation of five different, newly evolved copper resistant *Psa* strains revealed that while the bulk of the genome differed from the original *Psa* strain introduced into New Zealand by only 2 SNPs, the ICE_eno in several cases had moved from the att-1 site to a second, att-2, insertion site while ICEs harboring variants of copper resistance genes had replaced them at the att-1 site. In yet another copper-resistant strain, one of these same ICEs harboring copper resistance genes had inserted at the att-1 site with the original ICE_eno having moved to the att-2 site, while in yet another, an ICE harboring copper resistance genes had inserted into the att-2 site while ICE_eno had been evicted from the chromosome. To see such evidence of frequent intra-genomic movement of these insertion elements speaks to a highly dynamic process of ICE translocation leading to rapid chromosome evolution. The speed with which these novel ICEs invaded the apparently otherwise clonal *Psa* population and their instability within the genome is perhaps consistent with the apparent complex heritage of these ICEs conferring copper resistance. Core genes of the copper resistance-encoding ICEs in *Psa* are highly similar to those found not only elsewhere in New Zealand but also in related *Pseudomonas* strains from around the world. Likewise, copper resistance determinants found in the *Psa* ICEs also were quite similar to those found in other *Pseudomonas* species, again speaking to the apparent promiscuity of these elements. Studies by Colombi *et al.* (2017) showed that at least the one ICE conferring copper resistance in *Psa* that was investigated in some detail apparently imposed no detectable fitness costs on strains harboring it, either *in vitro* or *in planta*, yet

clearly conferred a strong fitness benefit in the presence of copper bactericides. While somewhat surprising, such a result would tend to explain why *Psa* was able to so quickly acquire such an ICE; while such ICEs would be expected to be common in bacterial communities subjected to the stress of copper bactericides, a lack of a fitness cost of their carriage would suggest that they may be common even in environments where selection for copper resistance is not present. Metagenomic studies of microbial communities to which plant pathogens may come into contact should provide valuable information on the likelihood with which such interspecific transfer will occur in a given setting. Transfer of an ICE conferring copper resistance to copper-resistant strains was also incredibly efficient, particularly on plants. Not only could transconjugants be detected within 30 min of co-culturing of such strains *in vitro*, but the frequency of transconjugant formation within three days together on leaves was as much as one per 50 recipient cells, and was also largely independent of the ratio of donor and recipient cells. While incredibly high rates of transconjugant formation was observed between *Psa* strains, much lower rates of ICE transfer into phylogenetically distant taxa such as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were observed. Earlier work had demonstrated that the rate of conjugal gene transfer on plants was particularly high, presumably due to relatively high microbial activity in such settings and favorable spatial proximity due to their growth together on plant surfaces (Normander *et al.*, 1998). The results of these studies therefore suggest that movement of ICEs through communities of taxonomically related strains on plants is to be expected and if the traits that they confer are of epidemiological significance, that we can anticipate further rapid evolution of emerging plant pathogens, some of which will prove difficult to control.

References

- Bartoli, C., Lamichhane, J.R., Berge, O., Guilbaud, C., Varvaro, L., Balestra, G.M., *et al.* (2015) A framework to gauge the epidemic potential of plant pathogens in environmental reservoirs: the example of kiwifruit canker. *Mol Plant Pathol* **16**: 137–149.
- Colombi, E., Straub, C., Kunzel, S., Templeton, M.D., McCann, H.C., and Rainey, P.B. (2017) Evolution of copper resistance in the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* through acquisition of integrative conjugative elements and plasmids. *Environ Microbiol* **19**: 819–832.
- McCann, H.C., Rikkerink, E.H.A., Bertels, F., Fiers, M., Lu, A., Rees-George, J., *et al.* (2013) Genomic analysis of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae* provides insight into the origins of an emergent plant disease. *PLoS Pathog* **9**: e1003503.

Morris, C.E., Sands, D.C., Viantzer, B.A., Glaux, C., Guilbaud, C., Buffiere, A., *et al.* (2008) The life history of the plant pathogen *Pseudomonas syringae* is linked to the water cycle. *ISME J* **2**: 321–334.

Normander, B., Christensen, B.B., Molin, S., and Kroer, N. (1998) Effect of bacterial distribution and activity on conjugal

gene transfer on the phylloplane of the bush bean (*Phaseolus vulgaris*). *Appl Environ Microbiol* **64**: 1902–1909.

Wozniak, R.A.F., and Waldor, M.K. (2010) Integrative and conjugative elements: Mosaic mobile genetic elements enabling dynamic lateral gene flow. *Nat Rev Microbiol* **8**: 552–563.