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The role of integrative conjugative elements in evolution of the kiwifruit pathogen *Pseudomonas syringae* pv. *actinidiae*

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Abstract

Horizontal gene transfer (HGT) is a major force driving evolution in prokaryotes. Among the different contributions to bacterial fitness, HGT underpins the evolution of pathogenicity and virulence in several bacterial pathogens. Pseudomonas syringae pv. actinidiae (Psa) first emerged as a pathogen of kiwifruit in Asia during the 1980's. In 2008 an outbreak occurred in Italy that rapidly spread to major kiwifruit growing areas of the world. During its global journey the outbreak lineage independently acquired divergent Integrative and Conjugative Elements (ICEs), harbouring an identical set of cargo genes that are hypothesized to be involved in the plant-pathogen interaction. Here I show that the three ICEs acquired by *Psa* belong to a diverse family of ICEs present only in plant-associated *Pseudomonas* ssp. (the PsICEs). The evolution of PsICEs is characterized by extensive inter-ICE recombination events that are frequent enough to mask evolutionary history, producing chimeras with variable patterns of similarity to each other, yet maintaining a syntenic backbone where cargo genes are integrated in conserved positions. Although there are different classes of PsICE cargo genes, one set was frequently recovered: those contained on a Tn6212 element. Tn6212 confers a selective benefit when *Psa* is grown on succinate, fumarate, or malate as the only carbon source, but no phenotype was detected in planta. Members of the PsICE family also confer copper resistance to Psa strains isolated in New Zealand. I analyzed a number of these and showed transfer in vitro and in *planta*. I also measured the fitness consequences of ICE carriage, captured the *de* novo formation of novel recombinant ICEs, and explored ICE host-range. Together my work, which began with observations from genome sequences before moving to experimental studies in the laboratory, has provided new insights into the role that horizontal gene transfer plays in the evolution of virulence.

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