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Prophage-mediated population dynamics of 'Candidatus Liberibacter asiaticus' in plant and insect hosts

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As an intracellular bacterium, 'Candidatus Liberibacter asiaticus' (Las) lacks known transposons and IS elements but contains at least two prophages/phages. In this study, we revealed the genetic diversity and population dynamics of this bacterium based on two prophage hypervariable regions (HVRs) using separate libraries constructed from citrus, periwinkle and psyllid. A total of 9 variants were identified, including 4 abundance types A, B, C, D and 5 rare types E, A1, A2, C1 and C2. The two HVRs, Type A and B, share highly conserved sequences and are localize to the two prophages, FP1 and FP2, respectively. The most abundant type in the psyllid library was Type A (36.71%), followed by Type B (25.17%) and Type C (19.72), but there was no Type D. However, the most abundant type in citrus was Type B (64.24%), followed by Type A (20.14%), C (11.11%) and D (1.39%). More interestingly, the Type A sequence was a very rare group (0.36%) in the periwinkle library. The most abundant type in the periwinkle library was Type B (43.73%) followed by Type C (39.07%) and then Type D (9.32%). Sequence analysis of these variants revealed the variations were due to the recombination and reassortment between two prophages. Conventional PCR results using primers specific to the different types indicated that Type A, B, C and D were present in more than 94.6% of higher titer Las-infected plant hosts; however, only 16.7% of tested psyllids contained D variants, which were very low titer, and Las-infected psyllids possessed higher titer Type A, B and C populations. Typing results for Las-infected citrus field samples indicated that only the Type D population was associated with huanglongbing (HLB) symptoms: high titer of D with typical blotchy mottle and extremely low to no type D with vein yellowing or other atypical HLB symptoms. Our finding that Las population dynamics derive from the prophage/phage activities may lead to a better understanding of how these bacteria evolve and adapt in different ecological niches.