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Plasmid genome dynamics of sequenced strains of *Xanthomonas campestris* pv. *campestris*

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Xanthomonas campestris pv. *campestris (Xcc)*, the causal agent of black rot of crucifers, remains a threat to all Brassica crops around the world for almost 140 years [3, 7, 9]. Bacteria of the genus *Xanthomonas* cause diseases on over 500 plant species [6]. Recent genomic and genetic data improved our understanding of *Xcc* taxonomy and virulence mechanisms. Genome sequences of 71 *X. campestris* strains have been published by December 2020 [2]. Each has ~ 65 % GC content and consists of a 4.9–5.2 Mb chromosome. Some strains have with up to 3 plasmids. A survey of 46 Xcc isolates from a global collection that included long-read sequencing showed that 29 strains encode 26 unique transcription activator-like effector (TALE) proteins [1], a class of type III effector (T3E) proteins translocated to host plant cells via type III secretion system (T3SS). *Xcc* strains have been found to contain between zero and four TALE genes found on the chromosome or on plasmids [1]. While TALEs play important role in many *Xanthomonas* pathogens, more research is needed to determine whether they act as virulence factors for *Xcc*. *Xcc* TALE genes are often found on plasmids and in association with mobile genetic elements. Plasmids in *Xanthomonas* species were associated with bacteria's adaptation to antibiotics, heavy metals, and to resistant host plants via horizonal transfer of virulence genes, including T3SS effector protein genes. When 117 strains representing 26 pathovars were examined for plasmid content and RFLP of plasmid DNAs, all tested strains of 10 pathovars contained plasmids, while all tested strains of 13 pathovars contained no detectable plasmids. Three pathovars varied in plasmid content. The apparent stability of the plasmids provides a natural genetic marker that can be strain-specific and useful in epidemiological investigations [5].
It was known that most of epidemic *Xcc* isolates do not have plasmids, and plasmids are not stably maintained in *Xcc* cells after artificial conjugation [9]. NCBI genebank has 125 genomes of *Xcc*, and only 18 (14 %) genomes includes plasmid sequences (*https://www.ncbi.nlm.nih.gov/genome/ browse/ #!/ plasmids/ 151/).* Size of the plasmids vary from 867 bp in Xcc CFBP 11247 to 147,727 bp in CFBP 4955. The smallest know *Xcc* plasmid (Genebank acc. *NZ_CM002634.1)* has transposase gene (family IS3) only. The largest known plasmid in *Xcc* CFBP 11247 (NZ_CM002552.1) is highly similar to plasmids in *Xcc* CN05 and CN13 (99.7–99.9 for 83–87 % of DNA length) and in *X. euvesicatoria* LMG930 (plasmid pLMG930.2), *X. euvesicatoria* 85-10 (plasmid p_XCV_1), and *X. campestris* pv. *vesicatoria* plasmid pXCV183 with 99.68 % identity for 83 % of sequence length. Remarkably, the putative type 4 secretion systems encoded on this plasmid is similar to the Icm/Dot systems of the human pathogens *Legionella pneumophila* and *Coxiella burnetii*. DotG/IcmE/VirB10 family protein on *Xcc* CFBP 11247 plasmid has homologues in a few other *Xcc* strains (Cf4B1, Cf3C, and

GBBC 1372) only, but commonly present in most of *X. euvesicatoria* strains. The type IV secretion system (T4SS) is known to play an important role in the conjugative transfer of genetic material, and also provides fitness to pathogenic bacteria for their enhanced survival [4]. Another group of large *Xcc* plasmids represented by MAFF106712 pXcc1 has T3E gene Xcc1_44040 (TAL group) and was found in many Xcc strains either as plasmid or chromosome part (Fig. 1).

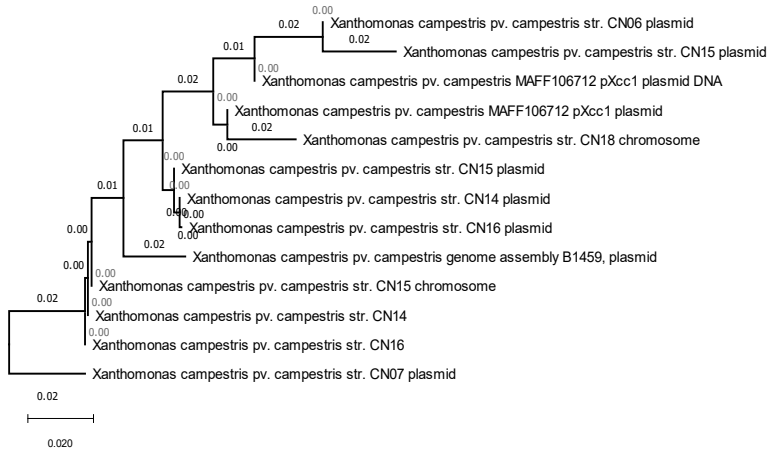

Fig. 1. Phylogenetic relations of sequences similar to complete genome of MAFF106712 pXcc1 plasmid in other *Xcc* strains

High level of inter-strain variation mediated by plasmids harboring virulence-related genes, including the T4SS and T3SS effectors highlights the importance of strain-specific genome dynamics mediated by such elements. Majority of epidemic *Xcc* strains sequenced to the date do not have plasmids but carry plasmid-homologues genes in their chromosomes. This suggests the importance of plasmid integration into bacterial chromosome via unknown so far mechanism in providing *Xcc* strains high virulence towards commercial brassicas.

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