

The Role of Domesticated Plasmids in The Plant-Associated Lifestyle of *Pantoea agglomerans*: A Genetic and Functional Investigation

Dissertation

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ABSTRACT

Many bacterial genomes reported to date have shown the presence of more than one replicon apart from the main chromosome. Understanding the functional advantages provided by such multipartite genome organization is key to uncovering the evolutionary origins of plasmids that serve as secondary replicons. Plasmids, often regarded as accessory genetic elements in bacteria, facilitate niche expansion by acquisition of beneficial traits. Most mobile plasmids are known to impose fitness costs upon invasion, but in the case of domesticated plasmids, plasmid-curing may lead to a negative fitness impact. This thesis presents two such domesticated plasmids called the large *Pantoea* plasmid (LPP) and the pPag1, which are widespread in the *Pantoea* genus and are proposed to be “chromids” (CHROmosomes of plasMID origin), a type of secondary replicon. We have previously observed that the curing of these plasmids negatively impacts root colonization in *Pantoea agglomerans* R1 (*Pantoea*). In this thesis, we investigate the functional roles of the LPP and pPag1 in *Pantoea*, focusing not only on their contribution to the bacterium’s metabolism and ability to colonize wheat roots but also on how their interaction with the chromosome shapes gene expression in the respective environments. We show that the loss of LPP negatively impacts the metabolic potential of *Pantoea*, which may be linked to its decreased growth and fitness in laboratory media or *in vitro*, highlighting the metabolic advantages conferred by the plasmid. Furthermore, by focusing on analyzing differences in gene expression of *Pantoea* and its plasmid-cured variants, both *in vitro* and during the early stages of plant colonization, we observe that the absence of LPP causes alteration of chromosomal gene expression in both environments. Using differential transcriptomics, statistical methods, functional category annotation, and extensive manual screening, we identify LPP-encoded genes potentially influencing chromosomal gene expression important for host association. This coordinated gene expression between the plasmid and chromosome in *Pantoea* suggests the involvement of inter-replicon communication in shaping the interaction of *Pantoea* with its wheat host. Thus, we infer that *P. agglomerans* exhibits an example of the evolution of plasmid-chromosome crosstalk, which facilitates a habitat switch in this plant-associated bacterium through gene regulation.