Plasmid 73 (2014) 16–25

Contents lists available at ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

Bigger is not always better: Transmission and fitness burden of ~1 MB *Pseudomonas syringae* megaplasmid pMPPla107

Artur Romanchuk [a,*], Corbin D. Jones [a], Kedar Karkare [a], Autumn Moore [b], Brian A. Smith [b], Chelsea Jones [b], Kevin Dougherty [b], David A. Baltrus [b]

[a] University of North Carolina – Chapel Hill, United States
[b] University of Arizona, United States

ARTICLE INFO

Article history:
Received 31 December 2013
Accepted 14 April 2014
Available online 2 May 2014
Communicated by Eva Top

Keywords:
Megaplasmid
HGT
Fitness cost
Plasmid transfer
Pseudomonas

ABSTRACT

Background: Horizontal gene transfer (HGT) is a widespread process that enables the acquisition of genes and metabolic pathways in single evolutionary steps. Previous reports have described fitness costs of HGT, but have largely focused on the acquisition of relatively small plasmids. We have previously shown that a *Pseudomonas syringae* pv. *lachrymans* strain recently acquired a cryptic megaplasmid, pMPPla107. This extrachromosomal element contributes hundreds of new genes to *P. syringae* and increases total genomic content by approximately 18%. However, this early work did not directly explore transmissibility, stability, or fitness costs associated with acquisition of pMPPla107.

Results: Here, we show that pMPPla107 is self-transmissible across a variety of diverse pseudomonad strains, on both solid agar and within shaking liquid cultures, with conjugation dependent on a type IV secretion system. To the best of our knowledge, this is the largest self-transmissible megaplasmid known outside of *Sinorhizobium*. This megaplasmid can be lost from all novel hosts although the rate of loss depends on medium type and genomic background. However, in contrast, pMPPla107 is faithfully maintained within the original parent strain (*Pla*107) even under direct negative selection during laboratory assays. These results suggest that *Pla*107 specific stabilizing mutations have occurred either on this strain's chromosome or within the megaplasmid. Lastly, we demonstrate that acquisition of pMPPla107 by strains other than *Pla*107 imparts severe (20%) fitness costs under competitive conditions *in vitro*.

Conclusions: We show that pMPPla107 is capable of transmitting and maintaining itself across multiple *Pseudomonas* species, rendering it one of the largest conjugative elements discovered to date. The relative stability of pMPPla107, coupled with extensive fitness costs, makes it a tractable model system for investigating evolutionary and genetic mechanisms of megaplasmid maintenance and a unique testing ground to explore evolutionary dynamics after HGT of large secondary elements.

1. Background

Horizontal gene transfer mediated by large secondary elements, which results in the movement of genomic regions directly between organisms without reproduction, substantially alters evolutionary dynamics within microbial populations (Gogarten and Townsend, 2005; Diaz-Ricci and Hernández, 2000). HGT facilitates rapid phenotypic evolution (Pennisi, 2004), the incorporation of new metabolic pathways and network expansions, and can lead to ecological speciation across microbes (Lawrence and

* Corresponding author.
E-mail address: romanchk@email.unc.edu (A. Romanchuk).

http://dx.doi.org/10.1016/j.plasmid.2014.04.002
0147-619X/

Roth, 1996; Shapiro et al., 2012; Lawrence and Ochman, 1998; Loper et al., 2012; Vogel et al., 1998). Similarly, HGT is a major mechanism behind the acquisition of antibiotic resistance genes and new virulence determinants, which facilitate the emergence of new pathogen types (Grad et al., 2012; Sørensen et al., 2005; Baltrus et al., 2011; Ashbolt et al., 2013; Broaders et al., 2013; Warnes et al., 2012).

Megaplasmids are the largest contiguous regions that can undergo HGT across bacterial cells. The term chromid has been proposed to designate a subset of megaplasmids that share a number of important characteristics with the main chromosome including similar GC content and suites of loci which are diverged from, yet potentially provide redundant functions to, existing housekeeping genes. These genomic patterns have led to the speculation that secondary chromosomes in genera such as *Burkholderia* and *Vibrio* began as chromids (Cooper et al., 2010), although distinct transitional forms have yet to be clearly identified. However, unlike the chromosome, chromids maintain plasmid replication and partitioning systems and the bulk of the genes that they carry may be neutral or serve accessory functions. Importantly, genus-specific genes are overrepresented on chromids and this lack of genus-independent genetic diversity suggests the existence of barriers to megaplasmid transfer outside of closely related species. Given that secondary chromosomes may be "evolutionary test beds" (Cooper et al., 2010) and chromids contain an abundance of unique genes and pathways, HGT of large secondary elements has the potential to dramatically alter evolutionary trajectories within microbial species (Wolf et al., 2012).

To be maintained at high frequencies within populations, regions transferred via HGT must provide fitness benefits, minimize fitness costs, or transfer to new host cells at high rates (Gomes et al., 2013; Ponciano et al., 2007). However, benefits of HGT are often partially counterbalanced by metabolic or physiological costs as natural selection, prior to acquisition, has not had an opportunity to fine tune interactions between transferred regions and their new genomic contexts (Baltrus et al., 2012). Previous studies have investigated both the positive and negative effects of HGT using relatively small plasmids (<15 kb) (De Gelder, 2008; Harrison and Brockhurst, 2012; Kwon et al., 2005; although see Platt et al., 2012), but much of this work provides an incomplete view as plasmids can range up to 2 Mb (Smillie et al., 2010). Because increasing plasmid size appears to be negatively correlated with self-transmissibility, and plasmids may fundamentally change in gene content and composition above an imprecisely defined threshold (Harrison et al., 2010; Harrison and Brockhurst, 2012), it remains unclear if and how selection pressures scale with plasmid size and how frequently such costs act as a barrier to horizontal transfer.

The megaplasmid pMPPla107 was first identified by genome sequencing of a phylogenetically diverse suite of *Pseudomonas syringae* strains (Baltrus et al., 2011). Phylogenetic data suggests that acquisition was the product of a relatively recent HGT event within *P. syringae* pv. *lachrymans* (Baltrus et al., 2011). This secondary element contains all the hallmarks of previously identified chromids, including the presence of many hypothetical proteins, tRNA genes, and potential duplicate versions of housekeeping genes similar to those in other *Pseudomonas* species (Table 4). Furthermore, self-transmissibility is suggested by the presence of a putative type IV secretion system that is most similar in sequence to *Legionella* Dot/Icm (Baltrus et al., 2011).

In this work we demonstrate that pMPPla107 is self-transmissible across a diverse range of Pseudomonads. We also show that, despite significant deleterious effects on growth, pMPPla107 can be stably maintained within new genomic backgrounds when exposed to a range of growth conditions. That presence of this megaplasmid in natural host strains is strongly correlated with slower growth *in vitro* and *in planta* compared to closely related strains that lack pMPPla107 (Baltrus et al., 2011), suggests that these costs have not been completely compensated for through evolution. Due to the size of pMPPla107 and shared characteristics with previously discovered chromids, this system provides a unique and experimentally tractable opportunity to study how large-scale HGT alters evolutionary dynamics within bacterial populations.

1.1. Box 1

1.1.1. Hypothesis

Plasmids are a mobile gene pool that can be accessed by multiple unrelated bacterial species. Megaplasmids comprise a rich reservoir of genes available for transfer across bacteria, but which, under certain conditions, may also significantly lower fitness of the recipient bacteria. The large size of pMPPla107 and the nature of genes it carries enables laboratory tests of a key hypothesis concerning megaplasmid transmission dynamics and maintenance: the major barrier to spread of pMPPla107 across viable hosts is its deleterious fitness impact on the host cell rather than transfer rates.

1.1.2. Approach

We quantify pMPPla107 transfer rates to several related *P. syringae* pathovars, *Pseudomonas stutzeri*, and *Escherichia coli*. We further quantify loss rate of pMPPla107 across several genetic backgrounds in different growth media types. Finally, we compare fitness impact of the pMPPla107 megaplasmid between close relative of the parent *P. syringae* strain and a divergent species, *P. stutzeri*.

2. Methods

2.1. Culture conditions

All experiments were carried out at 27 °C. Liquid and solid medium consisted of either low salt Lysogeny broth (LB) or M9 minimal medium, prepared according to Sambrook (2001), and supplemented with additional carbon source and antibiotics where appropriate. For growth of *P. stutzeri* strains, saltwater LB (SWLB) was used instead of LB as a growth medium (Sikorski et al., 1998). All liquid cultures were incubated on a rotary shaker (200 rpm). Antibiotics were used as necessary in the following