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Natural transformation-based foreign DNA acquisition in a *Ralstonia solanacearum mutS* mutant

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Abstract

Mutator strains with defective methyl-mismatch repair (MMR) systems have been shown to play an important role in adaptation of bacterial populations to changing and stressful environments. In this report, we describe the impact of *mutS*::aacC3-IV inactivation on foreign DNA acquisition by natural transformation in the phytopathogenic bacterium *Ralstonia solanacearum*. A *mutS* mutant of *R. solanacearum* exhibited 33- to 60-fold greater spontaneous mutation frequencies, in accordance with a mutator phenotype. Transformation experiments indicated that intra- and interspecific DNA transfers increased up to 89-fold. To assess horizontal gene transfer (HGT) from genetically modified plants to *R. solanacearum,* fitness of the mutator was first evaluated in soil and plant environments. Competitiveness was not modified after 61 days in soil and 8 days in tomato, and the progress of plant decay symptoms was similar to that of the wild-type strain. Despite its survival in soil and in planta, and the powerful capacities of HGT, *R. solanacearum* was not genetically transformed by transgenic plant DNA in a wide range of in vitro and in planta tests.

Keywords: Horizontal gene transfer; *mutS*; Recombination; *Ralstonia solanacearum*

1. Introduction

In addition to other mechanisms such as point mutations, horizontal gene transfer (HGT) among bacteria can lead to significant genetic modifications of bacterial genomes [1] and is therefore considered one of the main forces behind prokaryote evolution [29]. Three mechanisms have been reported to be involved in gene transfer: natural genetic transformation, conjugation and bacteriophage-mediated transduction [38]. Several studies have shown that environmental conditions such as the specific chemical composition of their medium [10], thermal shocks [2] or electrical parameters related to lightning discharges [9] could lead to the passive entry of DNA into bacterial cells, contributing to increasing the flow of incoming DNA. According to various reports and whatever the active or passive mechanism considered, DNA uptake is unlikely to be the major limitation step for HGT. In contrast, mechanisms controlling the integration of transforming DNA into the host genome could represent the main barrier for regulation of DNA acquisition and stable inheritance.

Two main and antagonistic systems, the methyl mismatch repair (MMR) system and the SOS system, regulate genetic stability by controlling the recombination-based integration process. The MMR system acts as the main barrier to recombination between divergent sequences, therefore limiting gene exchange among unrelated microorganisms [32]. Inactivation of the *Escherichia coli* MMR by disrupting the *mutS, mutL* or *mutH* genes leads to a mutator phenotype, increasing the potential for interspecies recombination events. An *E. coli*

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mutator exhibits a recombination rate with *Salmonella typhimurium* DNA increased by more than 3 orders of magnitude compared to the wild-type strain [32]. Some MMR system homologs have also been well-described, such as the HexAB mismatch repair system in *Streptococcus pneumoniae* [16]; however, their contributions to sexual isolation have been described as variable during interspecific HGT [22,25].

Interestingly, a significant proportion of pathogenic and commensal bacterial cells isolated from natural environments exhibit elevated mutation frequencies, including *E. coli* [21], *Pseudomonas aeruginosa* [30], *Streptococcus pneumoniae* [28], *Haemophilus influenzae* [34] and *Neisseria meningitidis* [33]. Most of these natural mutators have a defect in the MMR system, especially due to deletion [15,21,28,30] or large chromosomal inversion [19] into the *mutS* homologs.

Induction of the SOS response by stress factors (e.g. UV irradiation) also increases genetic plasticity by stimulating DNA rearrangements [12] and gene transfer [24]. These data suggest that bacteria can modulate the generation of genetic diversity and the maintenance of genetic integrity, optimizing the benefit of acquiring foreign genes.

In this paper, our objectives were to investigate the putative role of the *Ralstonia solanacearum* MMR system in the acquisition and recombination of new genetic information. The plant pathogen *R. solanacearum* GMI1000 (β-proteobacterium) was found to develop competence in vitro [5] and in plant tissue [3], leading to gene exchange under natural environmental conditions during the plant infection process. In their plant environment, *R. solanacearum* could come into contact with DNA from bacteria belonging to the same species and to different species or genera. The *R. solanacearum* genome exhibits a mosaic structure characterized by alternative codon usage regions (ACURs) with a G+C content and codon usage differing significantly from the rest of the genome (http://bioinfo.genopole-toulouse.prd.fr/annotation/iANT/bacteria/ralsto/) [35]. ACURs may result from numerous gene transfer and recombination events during *R. solanacearum* evolution, and their role in the evolution of virulence, host specificity or overall adaptation to environments such as soil and rhizosphere deserves to be investigated.

A *R. solanacearum* mutator strain was constructed by disrupting the *mutS* gene to determine the potential of a *mutS* mutant for intraspecies and interspecies bacterial gene transfer. Moreover, previous results showing the release of plant DNA [8] and the development of competence in *R. solanacearum* during plant infection [3] also led us to investigate the risk of dissemination of engineered genes from transgenic plants to mutator *R. solanacearum*.

2. Materials and methods

2.1. Bacterial strains and DNA

Bacterial strains and plant DNA used in this study are listed in Table 1. *E. coli* DH5α was used as a host for cloning of the different plasmids.

Table 1
Bacterial strains, plasmids and plants

Strain, plasmid and plant	Description/relevant genotype	References
Strains		
Escherichia coli		
DH5α	F⁻ *recA lac*ZΔM15	Invitrogen
Ralstonia solanacearum		
GMI1000	Wild-type strain. Phylotype I	[35]
GMIΔmutS	Δ*mutS*::*aac*C3-IV mutant from GMI1000	This work
GMI1581	*prhJ*::Ω mutant from GMI1000	[7]
CFBP734	Wild-type strain. Phylotype III	CFBP[a]
Plasmids		
pBluescript	Cloning vector	Stratagene
pGEM-T/ pGEM-T Easy	Cloning vector	Promega
pGPS3	Mutagenesis vector with *Apha-3* gene	Biolabs
pEFmutS	*mutS* PCR fragment inserted in pBluescript	This work
pEFΔmutS	Δ*mutS*::*aac*C3-IV	This work
pFtsk$_{1000}$01	*ftsK* PCR fragment from GMI1000 inserted in pGEM-T	This work
pFtsk$_{734}$01	*ftsK* PCR fragment from CFBP734 inserted in pGEM-T	This work
pFtsk$_{1000}$02	*ftsK*$_{1000}$::*aadA*	This work
pFtsk$_{734}$02	*ftsK*$_{734}$::*aadA*	This work
pZpop1	*popA*::*aadA*, *aac*C3-IV (*R. solanacearum* GMI1000)	[4]
pR23	23S PCR fragment from GMI1000 inserted in pGEM-T Easy	This work
pR23A3	23S::*aphA-3* (*R. solanacearum* GMI1000)	This work
pE23A3	23S::*aphA-3* (*E. coli* K12)	[37]
pP23A3	23S::*aphA-3* (*Pseudomonas putida* KT2440)	[37]
pA23A3	23S::*aphA-3* (*Acinetobacter calcoaceticus* ATCC 33604)	[37]
pBHC	Broad-host-range vector containing *rbcL* and *accD* from the tobacco plastid	[9]
Plants		
Lycopersicon esculentum		
Ailsa craig pKHG3	Nuclear transgenic; 1 copy of T-DNA from pKHG3	[27]
Ailsa craig Zpop1	Nuclear transgenic; 2 copies of T-DNA from pZpop1	[4]
Nicotiana tabacum		
cv. pBD6 LE01	Plastid transgenic; at most 10,000 copies of *aad*A flanked by *rbcL accD*	[17]

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2.2. Construction of the mutS::aac*C3-IV mutant*

The high degree of amino acid sequence conservation of MutS proteins in *E. coli* [36], *Azotobacter vinelandii* [20]