





Research in Microbiology 158 (2007) 537-544

www.elsevier.com/locate/resmic

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

Anne Mercier a,b, Franck Bertolla a,b,*, Eugénie Passelègue-Robe a,b, Pascal Simonet c

^a Université de Lyon, F-69003 Lyon, France

^b Ecologie Microbienne, CNRS, UMR 5557, INRA, USC 1193, Université Lyon I, F-69622 Villeurbanne, France

^c Environmental Microbial Genomic group, UMR CNRS 5005, Ampère, Ecole Centrale de Lyon, F-69134 Ecully Cedex, France

Received 19 April 2007; accepted 11 May 2007 Available online 27 May 2007

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.

© 2007 Elsevier Masson SAS. All rights reserved.

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

E-mail address: bertolla@biomserv.univ-lyon1.fr (F. Bertolla).

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*

^{*} Corresponding author. Ecologie Microbienne, UMR 5557, Université Lyon I, 43 bd du 11 Novembre 1918, F-69622 Villeurbanne, France. Tel.: +33 4 72 43 27 58; fax: +33 4 72 43 12 23.

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* DH5 α 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, FrecA lacZΔM15 Invitrogen Ralstonia solanacearum GMI1000 Wild-type strain. Phylotype I [35] GMI1000 GMI1581 ΔmutS::aacC3-IV mutant from GMI1000 [7] CFBP734 Wild-type strain. Phylotype III CFBP³ Plasmids pBluescript Cloning vector Stratagene PGEM-T Cloning vector Promega PGEM-T Cloning vector Promega PGEM-T Biolabs Apha-3 gene promega pEFAmutS Apha-3 gene This work pEFAmutS AmutS:aacC3-IV This work pEFAmutS AmutS:aacC3-IV This work pEFAmutS AmutS:aacC3-IV This work pFtsk ₁₀₀₀ 01 ftsK PCR fragment from CR CR CR CR This work CR	Bacterial strains, plasmids and plants			
Escherichia coli DH5α F recA lacZΔM15 Invitrogen Ralstonia solanacearum GMI1000 Wild-type strain. Phylotype I [35] GMI1000 GMI1000 [7] GMI1581 prhh::Ω mutant from GMI1000 [7] CFBP734 Wild-type strain. Phylotype III CFBP³ Plasmids pBluescript CCloning vector Stratagene pGEM-T Cloning vector Promega Easy pGEM-T Cloning vector Promega pEFMUS Mutagenesis vector with Apha-3 gene Biolabs Apha-3 gene Biolabs Apha-3 gene pEFMUS mutS PCR fragment inserted in pBluescript This work fragment from GMI1000 inserted in pGEM-T This work GMI1000 inserted in pGEM-T pFtsk100001 fisk PCR fragment from GMI1000 inserted in pGEM-T This work GMI1000 This work GMI1000 pR23 238 PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 inserted in pGEM-T Easy This work GMI1000 pR23A3 238::aphA-3 (R. solanacearum GMI1000) This work GMI1000 pB23A3 238::aphA-3 (R. solan	•	•	References	
DH5α F recA lacZΔM15 Invitrogen	Strains			
Ralstonia solanacearum GMI1000 Wild-type strain. Phylotype I [35] GMI1000 GMI1000 This work GMI1581 prhJ::Ω mutant from GMI1000 [7] CFBP734 Wild-type strain. Phylotype III CFBP³ Plasmids pBluescript Cloning vector Stratagene pGEM-T/PGEM-T Cloning vector Promega pGEM-T Easy PGEM-T Promega pEFMutS Apha-3 gene pEFDEMT This work pEFAmutS Apha-3 gene pEFAmutS PCR fragment inserted in pBluescript This work pFtsk100001 fisk PCR fragment from This work pFtsk100001 fisk PCR fragment from This work pFtsk100001 fisk PCR fragment from This work pFtsk3401 fisk Post. Fragment from This work pFtsk3402 fisk734::aaadA This work pFtsk100002 fisk1000::aaadA This work pFtsk100009 pR23 23S PCR fragment from This work pRMI10000 GMI1000 This work	Escherichia coli			
GMI1000 GMIΔmutS Wild-type strain. Phylotype I AmutS::aacC3-IV mutant from GMI1000 [35] This work GMI1000 GMI1581 CFBP734 Wild-type strain. Phylotype III CFBP³ Plasmids pBluescript pGEM-T/ pGEM-T Cloning vector Stratagene Promega pGPS3 Mutagenesis vector with Apha-3 gene pEFmutS Biolabs Apha-3 gene mutS PCR fragment inserted in pBluescript This work pBluescript pEFΔmutS pFtSh100001 ftsK PCR fragment from GMI1000 inserted in pGEM-T This work Pftsk73401 pFtsk73401 ftsK PCR fragment from CFBP734 inserted This work Pftsk73402 pFtsk100002 pFtsk73402 ftsK7000:aadA This work This work pZpop1 gopA::aadA, aacC3-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 pE3A3 23S::aphA-3 (R. solanacearum GMI1000 This work pP23A3 23S::aphA-3 (R. solanacearum GMI1000 [37] pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [9] PBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [4]	DH5α	F recA lacZΔM15	Invitrogen	
GMIΔmutS ΔmutS::aacC3-IV mutant from GMI1000 This work GMI1000 GMI1581 prhJ::Ω mutant from GMI1000 [7] CFBP734 Wild-type strain. Phylotype III CFBPa Plasmids Plasmids Stratagene pGEM-T/pGEM-T/pGEM-T Cloning vector Promega pGEM-T Easy Poper pGEM-T Easy Poper pEFmutS Mutagenesis vector with Apha-3 gene Biolabs Apha-3 gene pEFmutS mutS PCR fragment inserted in pBluescript This work Apha-3 gene pEFAmutS Δmuts::aacC3-IV This work GMI1000 inserted in pGEM-T pFtsk100001 ftsK PCR fragment from CFBP734 inserted in pGEM-T This work CFBP734 inserted in pGEM-T Easy pFtsk73402 ftsK734::aadA This work This work GMI1000 pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 pE23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000 pE23A3 23S::aphA-3 (Pseudomonas putida KT2440) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37]				
GMI1581				
CFBP734 Wild-type strain. Phylotype III CFBPa Plasmids pBluescript pGEM-T/ pGEM-T/ PGEM-T Easy pGPS3 Mutagenesis vector with Biolabs Apha-3 gene pEFmutS muS PCR fragment inserted in pBluescript pFtsM100001 ftsK PCR fragment from GMI1000 inserted in pGEM-T pFtsk100002 ftsK1000:aadA This work pFtsk73402 ftsK734:aadA acC3-IV pFtsk100002 ftsK734:aadA acC3-IV pFtsk100002 ftsK1000:aadA This work pFtsk73402 ftsK734:aadA acC3-IV pR23 23S PCR fragment from GMI1000 inserted in pGEM-T pR23 23S PCR fragment from This work GMI1000 inserted in pGEM-T Easy pR23A3 23S:aphA-3 (R. solanacearum GMI1000) pR24 23S:aphA-3 (R. solanacearum GMI1000) pB23A3 23S:aphA-3 (R. solanacearum GMI1000) pB23A3 23S:aphA-3 (R. solanacearum GMI1000) pB23A3 23S:aphA-3 (R. coli K12) [37] pP23A3 23S:aphA-3 (R. coli K12) [37] pP23A3 23S:aphA-3 (R. coli K12) [37] pP23A3 23S:aphA-3 (Acinetobacter calcoaceaticus ATCC 33604) pBHC Broad-host-range vector [9] PBHC Broad-host-range vector [9] Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17]			This work	
Plasmids pBluescript pGEM-T/ pGEM-T/ pGEM-T/ pGEM-T Cloning vector promega Stratagene promega pGEM-T pGEM-T pGEM-T Easy Poper Sa pene Mutagenesis vector with pBiolabs Apha-3 gene Biolabs Apha-3 gene pEFmutS pEFAmutS pEFAmutS pEFAmutS pFtsk100001 pfsk PCR fragment inserted in pBluescript pEFAmutS pFtsk100001 pfsk PCR fragment from pGM11000 inserted in pGEM-T pFtsk73401 pfsk PCR fragment from pGEM-T This work pFtsk73401 pfsk PCR fragment from pGEM-T pFtsk100002 pfsk PGM-T psk PG	GMI1581			
pBluescript pGEM-T/ Cloning vector Promega pGEM-T/ pGEM-T Easy pGPS3 Mutagenesis vector with Apha-3 gene pEFmutS mutS PCR fragment inserted in pBluescript pEFΔmutS ΔmutS::aacC3-IV This work pFtsk100001 ftsK PCR fragment from GMI1000 inserted in pGEM-T pFtsk73401 ftsK pCR fragment from CFBP734 inserted in pGEM-T pFtsk100002 ftsK1000::aadA This work pFtsk3402 ftsK34::aadA This work pFtsk3402 ftsK734:aadA This work pFtsk73402 ftsK734:aadA This work gMI1000) pR23 23S PCR fragment from GMI1000) pR24 23S PCR fragment from This work gMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) pB23A3 23S::aphA-3 (R. solanacearum GMI1000) pB23A3 23S::aphA-3 (R. solanacearum GMI1000) pB23A3 23S::aphA-3 (Acinetobacter GMI1000) pB23A3 23S::aphA-3 (Pseudomonas GMI1000) pB42A3A3 23S::aphA-3 (Pseudomonas GMI		Wild-type strain. Phylotype III	CFBP ^a	
pGEM-T/pGEM-T Cloning vector Promega Easy pGPS3 Mutagenesis vector with Apha-3 gene Biolabs Apha-3 gene pEFmutS mutS PCR fragment inserted in pBluescript This work of		·	_	
pGEM-T Easy pGPS3 Mutagenesis vector with Apha-3 gene Biolabs pEFmutS mutS PCR fragment inserted in PBluescript This work pBluescript pEFΔmutS ΔmutS::aacC3-IV This work pFtsk.100001 pFtsk.100001 ftsK PCR fragment from GMI1000 inserted in pGEM-T This work pFtsk.73401 pFtsk.73401 ftsK.76000:aadA This work pFtsk.73402 pFtsk.73402 ftsK.734:aadA This work pFtsk.73402 pFtsk.73402 ftsK.734:aadA This work pFtsk.73402 pZpop1 popA::aadA, aacC3-IV [4] (R. solanacearum GMI1000) This work GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pE23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [9] Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [4] [4] <t< td=""><td></td><td></td><td>-</td></t<>			-	
Easy pGPS3	•	Cloning vector	Promega	
pGPS3 Mutagenesis vector with Apha-3 gene Biolabs Apha-3 gene pEFmutS mutS PCR fragment inserted in pBluescript This work pBluescript pEFΔmutS ΔmutS::aacC3-IV This work of GMI1000 inserted in pGEM-T pFtsk100001 ftsK PCR fragment from CFBP734 inserted in pGEM-T This work CFBP734 inserted in pGEM-T pFtsk100002 ftsK1000::aadA This work QFTsk73402 ftsK734::aadA A This work QMI1000 This work QMI1000 pZpop1 popA::aadA, aacC3-IV (R. solanacearum GMI1000) [4] This work GMI1000 pR23 235 PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 This work GMI1000 pE23A3 235::aphA-3 (R. solanacearum GMI1000) This work GMI1000 This work GMI1000 pE23A3 235::aphA-3 (R. coli K12) [37] [37] [37] pBA23A3 235::aphA-3 (R. solanacearum GMI1000) [37] [37] [37] [37] pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [37] [37] PBHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] [4] Zpop1 2 copies of T-DNA from pZpop1	•			
Apha-3 gene pEFmutS mutS PCR fragment inserted in pBluescript This work pBluescript pEFΔmutS ΔmutS::aacC3-IV This work pFtsk100001 ftsK PCR fragment from GMI1000 inserted in pGEM-T This work pFtsk73401 ftsK PCR fragment from CFBP734 inserted in pGEM-T This work pFtsk100002 ftsK 1000:aadA This work pFtsk73402 ftsK734::aadA This work pZpop1 popA::aadA, aacC3-IV [4] [4] (R. solanacearum GMI1000) This work pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000 inserted in pGEM-T Easy pP23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pE23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pP23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [9] pSHG3 1 copy of T-DNA from pKHG3 <	•	Mutaganasis vactor with	Riolahe	
pEFmutS mutS PCR fragment inserted in pBluescript This work pBluescript pEFΔmutS ΔmutS::aacC3-IV This work pFtsk100001 pFtsk100001 ftsK PCR fragment from GMI1000 inserted in pGEM-T This work pGM1000 pFtsk73401 ftsK PCR fragment from CFBP734 inserted in pGEM-T This work pFtsk73402 pFtsk100002 ftsK1000::aadA This work pFtsk73402 pFtsk73402 ftsK734::aadA This work pFtsk73402 pFtsk73402 ftsK734::aadA This work pFtsk73402 pFtsk73402 ftsK724::aadA This work pFtsk73402 pFtsk73402 ftsK724::aadA This work pFtsk73402 pFtsk73402 ftsK72440A This work pFtsk73402 pR23 23S PCR fragment from pGMI1000 inserted prophytical prophyti	por 33	_	Diolaus	
pEFΔmutS ΔmutS::aacC3-IV This work pFtsk100001 ftsK PCR fragment from This work GMI1000 inserted in pGEM-T pFtsk73401 ftsK PCR fragment from This work CFBP734 inserted in pGEM-T pFtsk100002 ftsK1000:aadA This work pFtsk73402 ftsK734:aadA This work pZpop1 popA::aadA, aacC3-IV [4] (R. solanacearum GMI1000) This work pR23 23S PCR fragment from This work GMI1000 This work GMI1000 inserted This work pR23A3 23S::aphA-3 (R. solanacearum This work GMI10000 FE23A3 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 Plants Lycopersicon esculentum [4] Ailsa craig Nuclear transgenic; pKHG3 [4] Ailsa craig <td>pEFmutS</td> <td>. •</td> <td>This work</td>	pEFmutS	. •	This work	
pEFΔmutS ΔmutS::aacC3-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 ₇₃₄ 02 ftsK ₁₀₀₀ ::aadA inserted in pGEM-T This work pEtsk ₇₃₄ 02 ftsK ₇₃₄ ::aadA inserted in pGEM-T gament from GMI1000) [4] pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pE23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pBHC Broad-host-range vector containing rbcL and accO from the tobaccor plastid [4] Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4]	p21 maio		Tino work	
pFtsk ₁₀₀₀ 01 ftsK PCR fragment from GMI1000 inserted in pGEM-T This work GMI1000 inserted in pGEM-T pFtsk ₇₃₄ 01 ftsK PCR fragment from CFBP734 inserted in pGEM-T pFtsk ₁₀₀₀ 02 ftsK ₁₀₀₀ ::aadA This work This work This work Ptsk ₇₃₄ :02 pFtsk ₇₃₄ 02 ftsK ₇₃₄ ::aadA This work This work GMI1000) pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000) pE23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pP23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pBHC Broad-host-range vector coluctions ATCC 33604) [37] pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [9] Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 <td< td=""><td>pEF∆mutS</td><td></td><td>This work</td></td<>	pEF∆mutS		This work	
$\begin{array}{c} \text{in pGEM-T} \\ p\text{Ftsk}_{734}01 & \textit{ftsK} \ \text{PCR} \ \text{fragment from} \\ \text{CFBP734 inserted} \\ \text{in pGEM-T} \\ \\ p\text{Ftsk}_{1000}02 & \textit{ftsK}_{1000}::aadA \\ p\text{Ftsk}_{734}02 & \textit{ftsK}_{734}::aadA \\ \text{pZpop1} & \textit{popA}::aadA, aac\text{C3-IV} \\ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{R23} & 23\text{S PCR} \ \text{fragment from} \\ \text{GMI1000 inserted} \\ \text{in pGEM-T Easy} \\ \\ p\text{R23A3} & 23\text{S}::aphA-3 \ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{E23A3} & 23\text{S}::aphA-3 \ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{E23A3} & 23\text{S}::aphA-3 \ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{B23A3} & 23\text{S}::aphA-3 \ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{B23A3} & 23\text{S}::aphA-3 \ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{B23A3} & 23\text{S}::aphA-3 \ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{B23A3} & 23\text{S}::aphA-3 \ (\textit{R. solanacearum} \\ \text{GMI1000}) \\ \\ p\text{B23A3} & 23\text{S}::aphA-3 \ (\textit{Acinetobacter} \\ \text{calcoaceticus} \\ \text{ATCC 33604}) \\ \\ p\text{BHC} & \text{Broad-host-range vector} \\ \text{containing } \textit{rbcL} \ \text{and} \\ \text{accD} \ \text{from the tobacco plastid} \\ \\ P\text{lants} \\ \\ \textit{Lycopersicon esculentum} \\ \text{Ailsa craig} & \text{Nuclear transgenic;} \qquad [27] \\ \text{pKHG3} & 1 \ \text{copy of T-DNA from} \\ \text{pKHG3} \\ \text{Ailsa craig} & \text{Nuclear transgenic;} \qquad [4] \\ \\ \text{Zpop1} & 2 \ \text{copies of T-DNA from} \\ \text{pZpop1} \\ \\ \textit{Nicotiana tabacum} \\ \text{cv. pBD6 LE01} & \text{Plastid transgenic;} \qquad [17] \\ \text{at most } 10,000 \ \text{copies of} \\ \\ \end{array}$		ftsK PCR fragment from	This work	
pFtsk ₇₃₄ 01 ftsk PCR fragment from CFBP734 inserted in pGEM-T This work pEM-T pFtsk ₁₀₀₀ 02 ftsk ₁₀₀₀ ::aadA This work pEtsk ₇₃₄ :2adA pFtsk ₇₃₄ 02 ftsk ₇₃₄ ::aadA This work pEtsk ₇₃₄ :2adA pZpop1 popA::aadA, aacC3-IV (R. solanacearum GMI1000) [4] pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pE23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] p23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pA23A3 23S::aphA-3 (R. solanacearum GMI1000) [37] pBHC Broad-host-range vector colacoaceticus ATCC 33604) [37] pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [9] Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01		GMI1000 inserted		
CFBP734 inserted in pGEM-T pFtsk $_{1000}$ 02 $ftsK_{1000}$::aadA This work pFtsk $_{734}$ 02 $ftsK_{734}$::aadA, aacC3-IV [4] $(R. solanacearum GMI1000)$ pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pE23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pP23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pE23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pP23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pP23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pA23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pA23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pA23A3 23S::aphA-3 $(R. solanacearum GMI1000)$ pBHC Broad-host-range vector [9] containing $(R. solanacearum GMI1000)$ pBHC Broad-host-range vector [9] containing $(R. solanacearum GMI1000)$ pBHC Broad-host-range vector [9] containing $(R. solanacearum GMI1000)$ pSHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 $(R. solanacearum GMI1000)$ Plastid transgenic; [17] at most 10,000 copies of		in pGEM-T		
$\begin{array}{c} \text{in pGEM-T} \\ \text{pFtsk}_{1000}02 & ftsK_{1000}::aadA & \text{This work} \\ \text{pFtsk}_{734}02 & ftsK_{734}::aadA & \text{This work} \\ \text{pZpop1} & popA::aadA, aacC3-IV & [4] \\ & (R. solanacearum & GMI1000) \\ \text{pR23} & 23S PCR fragment from & \text{This work} \\ & GMI1000 \text{ inserted} & \text{in pGEM-T Easy} \\ \text{pR23A3} & 23S::aphA-3 (R. solanacearum & GMI1000) \\ \text{pE23A3} & 23S::aphA-3 (E. coli K12) & [37] \\ \text{pP23A3} & 23S::aphA-3 (Pseudomonas & [37] \\ putida KT2440) \\ \text{pA23A3} & 23S::aphA-3 (Acinetobacter & [37] \\ & calcoaceticus & \\ \text{ATCC } 33604) \\ \text{pBHC} & \text{Broad-host-range vector} & [9] \\ \text{containing } rbcL \text{ and} & \\ accD \text{ from the tobacco plastid} \\ \\ Plants & \\ Lycopersicon esculentum & \\ \text{Ailsa craig} & \text{Nuclear transgenic;} & [27] \\ \text{pKHG3} & 1 \text{ copy of T-DNA from pKHG3} \\ \text{Ailsa craig} & \text{Nuclear transgenic;} & [4] \\ \text{Zpop1} & 2 \text{ copies of T-DNA from pZpop1} \\ \\ Nicotiana tabacum & \\ \text{cv. pBD6 LE01} & \text{Plastid transgenic;} & [17] \\ \text{at most } 10,000 \text{ copies of} \\ \end{array}$	pFtsk ₇₃₄ 01	ftsK PCR fragment from	This work	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$		CFBP734 inserted		
pFtsk ₇₃₄ (02 ftsK ₇₃₄ ::aadA This work pZpop1 popA::aadA, aacC3-IV [4] (R. solanacearum GMI1000) This work 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 (R. solanacearum GMI1000) [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 Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17] at most 10,000 copies of [17]		in pGEM-T		
pZpop1 popA::aadA, aacC3-IV (R. solanacearum GMI1000) pR23 23S PCR fragment from This work GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) pE23A3 23S::aphA-3 (E. coli K12) [37] pP23A3 23S::aphA-3 (Pseudomonas [37] putida KT2440) pA23A3 23S::aphA-3 (Acinetobacter [37] calcoaceticus ATCC 33604) pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17]	=			
(R. solanacearum GMI1000) pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) pE23A3 23S::aphA-3 (E. coli K12) pP23A3 23S::aphA-3 (Pseudomonas putida KT2440) pA23A3 23S::aphA-3 (Acinetobacter calcoaceticus ATCC 33604) pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig pKHG3 Ailsa craig Nuclear transgenic; pKHG3 Ailsa craig Nuclear transgenic; [27] Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17] at most 10,000 copies of	· · · · · · · · · · · · · · · · · ·			
PR23 23S PCR fragment from This work	pZpop1		[4]	
pR23 23S PCR fragment from GMI1000 inserted in pGEM-T Easy This work GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000) 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 [27] Ailsa craig Nuclear transgenic; pKHG3 [27] Ailsa craig Nuclear transgenic; pKHG3 [4] Ailsa craig Nuclear transgenic; pzpp1 [4] Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; plastid tra		,		
GMI1000 inserted in pGEM-T Easy pR23A3 23S::aphA-3 (R. solanacearum This work GMI1000) pE23A3 23S::aphA-3 (E. coli K12) [37] pP23A3 23S::aphA-3 (Pseudomonas [37] putida KT2440) pA23A3 23S::aphA-3 (Acinetobacter [37] calcoaceticus ATCC 33604) pBHC Broad-host-range vector [9] containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17] at most 10,000 copies of [17]	D22		TIL:	
in pGEM-T Easy pR23A3	pR23	2	THIS WOLK	
pR23A3 23S::aphA-3 (R. solanacearum GMI1000) This work GMI1000) 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 Nuclear transgenic; pKHG3 [27] Ailsa craig Nuclear transgenic; pKHG3 [4] Ailsa craig Nuclear transgenic; p2pop1 [4] Nicotiana tabacum pZpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum pLastid transgenic; at most 10,000 copies of [17]				
GMI1000 pE23A3 23S::aphA-3 (E. coli K12) [37] pP23A3 23S::aphA-3 (Pseudomonas [37] putida KT2440 pA23A3 23S::aphA-3 (Acinetobacter [37] calcoaceticus [4] calcoaceticus [4] calcoaceticus [27] pBHC Broad-host-range vector [9] containing rbcL and accD from the tobacco plastid Plants [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum [17] calcoaceticus [18] [18] [18] calcoaceticus [18]	nR23A3	•	This work	
pE23A3 23S::aphA-3 (E. coli K12) [37] pP23A3 23S::aphA-3 (Pseudomonas) [37] putida KT2440) pA23A3 23S::aphA-3 (Acinetobacter calcoaceticus [37] ATCC 33604) pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid [9] Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum [7] cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of [17]	p1023/13	*	This work	
pP23A3 23S::aphA-3 (Pseudomonas putida KT2440) pA23A3 23S::aphA-3 (Acinetobacter calcoaceticus ATCC 33604) pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17] at most 10,000 copies of	pE23A3	· · · · · · · · · · · · · · · · · · ·	[37]	
putida KT2440) pA23A3 23S::aphA-3 (Acinetobacter [37] calcoaceticus ATCC 33604) pBHC Broad-host-range vector [9] containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17] at most 10,000 copies of	•	*		
calcoaceticus ATCC 33604) pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [27] pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of	1	putida KT2440)		
ATCC 33604) pBHC Broad-host-range vector [9] containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of	pA23A3	23S::aphA-3 (Acinetobacter	[37]	
pBHC Broad-host-range vector containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of		calcoaceticus		
containing rbcL and accD from the tobacco plastid Plants Lycopersicon esculentum Ailsa craig Nuclear transgenic; [27] pKHG3		ATCC 33604)		
Plants Lycopersicon esculentum Ailsa craig pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of	pBHC	_	[9]	
Plants Lycopersicon esculentum Ailsa craig pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [27] pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of		=		
Lycopersicon esculentum Ailsa craig Nuclear transgenic; pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of		accD from the tobacco plastid		
Ailsa craig Nuclear transgenic; [27] pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of				
pKHG3 1 copy of T-DNA from pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of			[27]	
pKHG3 Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of	· ·		[27]	
Ailsa craig Nuclear transgenic; [4] Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of	pKHO3			
Zpop1 2 copies of T-DNA from pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; at most 10,000 copies of [17]	Ailsa craig	*	[4]	
pZpop1 Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17] at most 10,000 copies of		-		
Nicotiana tabacum cv. pBD6 LE01 Plastid transgenic; [17] at most 10,000 copies of	• •			
at most 10,000 copies of	Nicotiana tabacum			
	cv. pBD6 LE01		[17]	
aadA flanked by rbcL accD				
		aadA flanked by rbcL accD		

^a Collection Française de Bactéries Phytopathogènes, Angers, France.

2.2. Construction of the mutS::aacC3-IV mutant

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

Download English Version:

https://daneshyari.com/en/article/4359480

Download Persian Version:

https://daneshyari.com/article/4359480

Daneshyari.com