A STABLE SHUTTLE VECTOR FOR *XYLELLA FASTIDIOSA* BASED ON AN ENDOGENOUS INCP-1 PLASMID

Principal Investigator:
Drake C. Stenger
USDA, ARS
Parlier, CA 93648
drake.stenger@ars.usda.gov

Co-Principal Investigators:
Min Woo Lee
USDA, ARS
Parlier, CA 93648
minwoo.lee@ars.usda.gov

Elizabeth Rogers
USDA, ARS
Parlier, CA 93648
elizabeth.rogers@ars.usda.gov

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ABSTRACT
Xylella fastidiosa (*Xf*) strain RIV11 harbors a 25 kbp plasmid (pXF-RIV11) belonging to the incP1 incompatibility group. Replication and stability factors of pXF-RIV11 were identified and used to construct plasmids able to propagate in both *Xf* and *Escherichia coli*. Sequences required for replication in *E. coli* and conferring antibiotic resistance were derived from the cloning vector pCR2.1. Replication in *Xf* required a 1.4 kbp region from pXFRIV11 containing a replication initiation gene (*trfA*) and the adjacent origin of DNA replication (*ori*V). This region also conferred plasmid replication in *Agrobacterium tumefaciens*, *Xanthomonas campestris*, and *Pseudomonas syringae*. Constructs containing the *trfA* gene and *ori*V derived from pVEIS01, a similar 31 kbp incP1 plasmid of the earthworm symbiont *Verminephropbacter eiseniae*, also were competent for replication in *Xf*. As expected, constructs bearing only *trfA* or *ori*V from either incP1 plasmid were unable to replicate in *Xf*. Although these incP1 replicons could be maintained in *Xf* under antibiotic selection, removal of selection resulted in loss of the plasmid. A novel toxin/antitoxin (*pem*I/*pem*K) addiction system of pXFRIV11 conferred stability of incP1 replicons in *Xf* in the absence of antibiotic selection. The resulting 6 kbp *Xf* shuttle vector (pXF20-PEMIK) also contains 10 unique endonuclease recognition sites for insertion of foreign DNA.

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