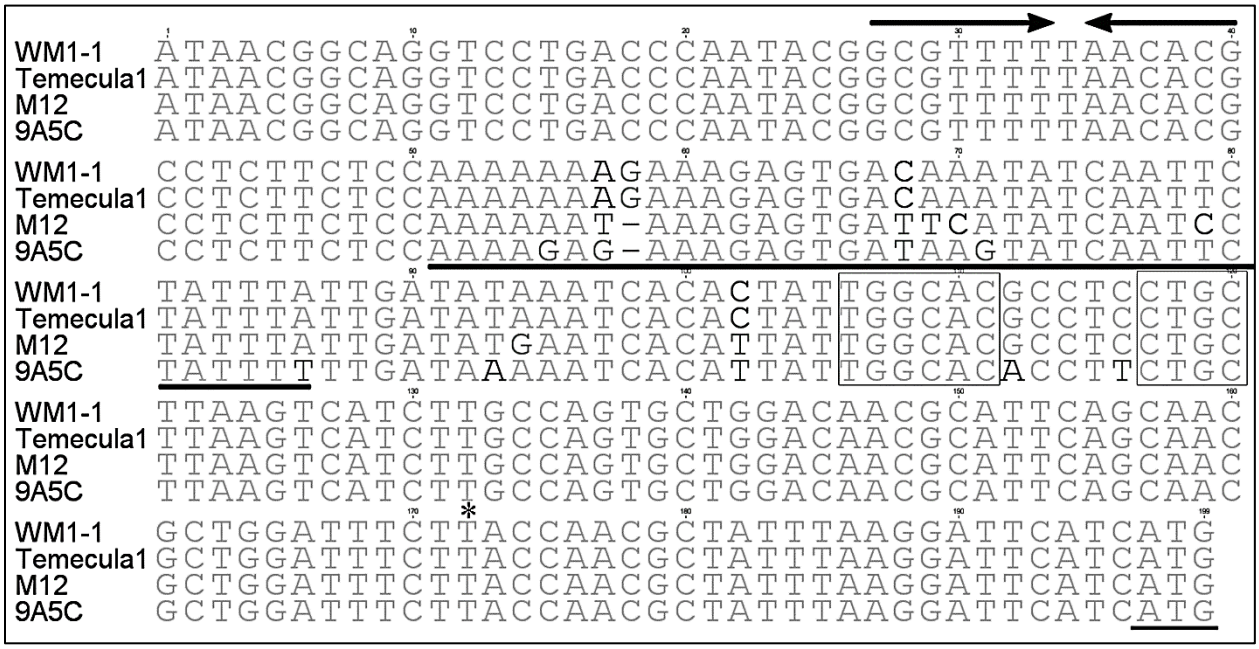
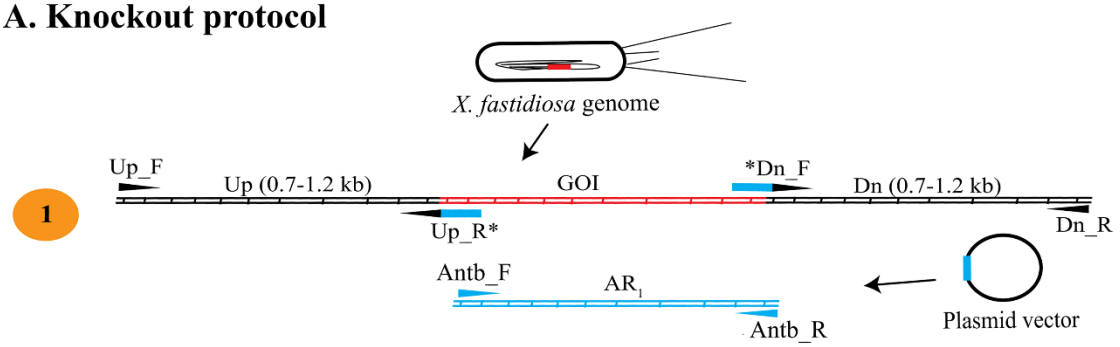


Fig. S1 Determination of characteristic features of a σ^{54} binding sites in the promoter of *PilA2* (PD1926) of *Xylella fastidiosa* subspecies *fastidiosa* strains WM1-1 and Temecula1. *X. f.* subsp. *multiplex* strain M12 and subsp. *pauca* strain 9a5c are included for comparison. Translation start site (ATG), and integration host factor binding sites are underlined. Asterisk (*) indicates the conserved transcription start site that is 66bp upstream of the translation start site predicted previously for strain 9a5c. Boxed regions indicate conserved elements of -12 (CTGC) and -24 (TGGCAC) regions of a typical σ^{54} factor. Arrows indicate conserved inverted repeats, the possible enhancer binding sites of the σ^{54} promoter.

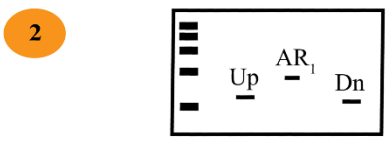
Fig. S2 Diagram of mutagenesis protocol. The protocols used here for knockout (A) and complementation (B) mutations are summarized in this diagram. For more details see Materials and Methods section.

A. Knockout protocol

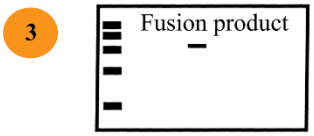


Primer design to amplify upstream (Up) and downstream (Dn) flanking regions of the gene of interest (GOI) from *X. fastidiosa* genome, and an antibiotic resistant marker (AR_1) from a plasmid vector

*Primers Up_R and Dn_F contain 21-27 bp 5' overhang homologous with AR_1 to promote overlap extension

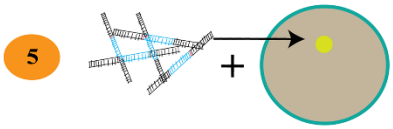


Independent PCRs of three fragments and gel purification

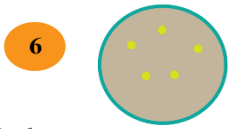


Overlap extension PCR to generate fusion product ($Up+AR_1+Dn$) and gel purification

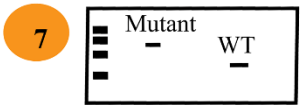
PCR amplification of the gel purified fusion product with internal primers



Natural transformation by mixing 10 µl of the gel-purified fusion product (3) or internal PCR product (4) with 10 µl of *X. fastidiosa* cells (OD_{600} 0.25) on a PD3 agar plate and incubation for 3 days at 28°C

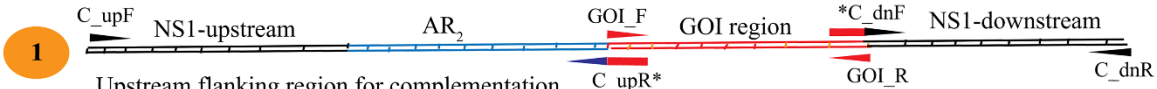


Selection of antibiotic resistant mutants by culturing in PW agar containing antibiotics



Confirmation of mutants by PCR and sequencing

B. Complementation protocol



Upstream flanking region for complementation containing antibiotic resistant marker 2 (AR_2, different from the one used in A). This region is amplified from a previously constructed plasmid vector (Matsumoto et al, 2009)

Gene of interest containing its coding region, promoter, and terminator

Downstream flanking region for complementation

NS1 is a neutral site previously identified in the genome of *X. fastidiosa* Temecula1 (Matsumoto et al, 2009)

2 Follow protocol A from step 2 to 7. In step 5, use the the mutant of GOI as recipient and in step 6 use both antibiotics to select for the complemented strains

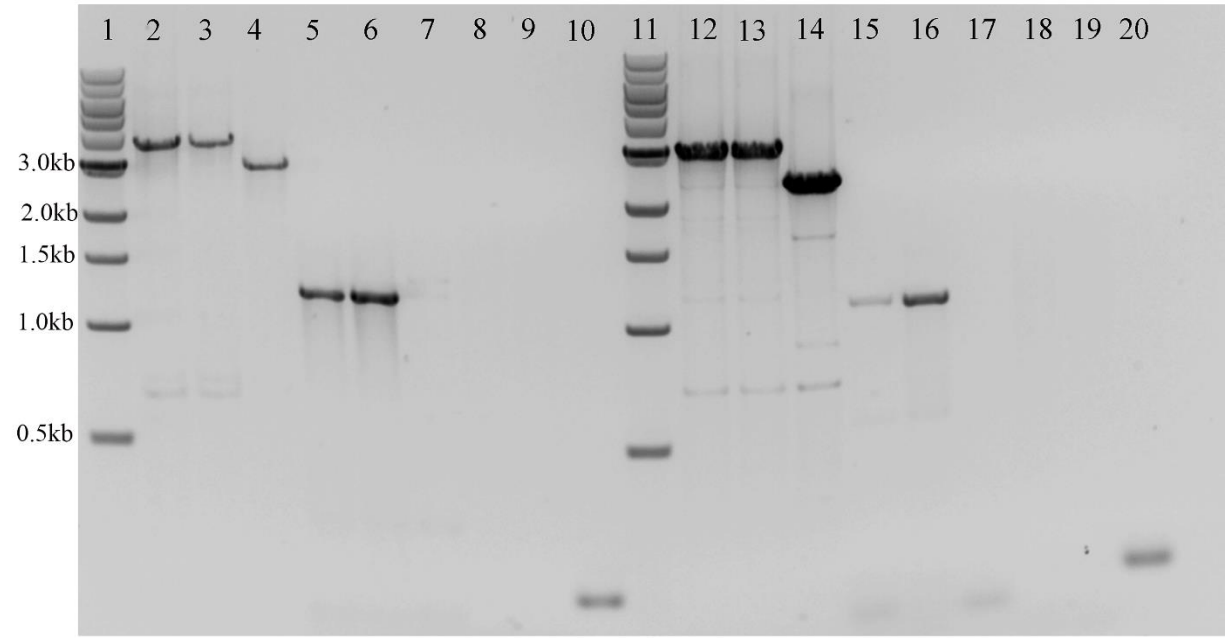


Fig. S3 Confirmation of *pilA1* (lanes 1-10) and *pilA2* (lanes 11-20) deletion from *Xylella fastidiosa* strains WM1-1 and TemeculaL. Three separate PCRs (out-, antibiotic, *pilA*) were performed to confirm each mutation. Out PCR targeted the region flanking the insertion site of the homologous template, antibiotic PCR targeted the antibiotic marker region, and *pilA* PCR targeted the coding region of each *pilA* paralogs. For each PCR, first and second lanes used DNA templates of the WM1-1 and TemeculaL mutants, respectively and the third lane used DNA template from wild-type WM1-1. Lanes 1 and 11 1kb ladder, lanes 2-4 *pilA1* out PCR, lanes 5-7 antibiotic PCR for *pilA1* (Kanamycin), lanes 8-10 *pilA1* PCR for *pilA1* coding region, lanes 12-14 *pilA2* out PCR, lanes 15-17 antibiotic PCR for *pilA2* (chloramphenicol), lanes 18-20 *pilA2* PCR for *pilA2* coding region. Longer PCR fragment from out-PCR in the mutants than in the wild-type suggest insertion of antibiotic cassettes, which is confirmed by the presence of bands with antibiotic PCR in mutants but not in wild-type. Amplification with the *pilA* specific PCR shows that the mutants lack the target genes and therefore no amplification, but the wild-type have intact gene therefore there is amplification (refer to Table 2 for amplicon sizes).

References for supplemental material:

Matsumoto A, Young GM, Igo MM. 2009. Chromosome-based genetic complementation system for *Xylella fastidiosa*. Appl Environ Microbiol **75:**1679-1687.