

Supporting Information

Killiny and Almeida 10.1073/pnas.0908562106

SI Materials and Methods

Insect Source and Maintenance. Adult blue-green sharpshooter leafhoppers (BGSS), *Graphocephala atropunctata* (Signoret) (Hemiptera, Cicadellidae), were collected on riparian vegetation at Russian river in Guerneville, Sonoma County, California. We reared insects on sweet basil. We used second-generation adults to perform the transmission test, as *X. fastidiosa* is not transstadially or transovarially transmitted. To confirm that the insects were *X. fastidiosa* free, we caged leafhoppers on healthy grape seedlings (*Vitis vinifera* cv. Cabernet Sauvignon) for 4 days before assays; none of these plants became infected. General insect rearing, plant maintenance, and handling protocols followed previously published work (1–2).

EPS Quantification. *Xylella fastidiosa* cells grown for 10 days on different solid media were suspended and adjusted to concentration of 10^8 CFU/mL (OD_{600} of 0.25), or we first washed cells with PBS and then adjusted suspensions to same concentration. To quantify EPS, we used a protein A double-antibody sandwich ELISA as described by ref. 3.

Afimbrial Adhesins (Hxfs) Quantification. We prepared 10-fold serial dilutions of a 10^8 CFU/mL (OD_{600} of 0.25) suspension of cells grown for 10 days on solid media. Dilutions were done in Tris-buffered saline (TBS), pH 7.4 (50 mM Tris-HCl at pH 7.4, 150 mM NaCl); 2 μ L of each dilution was dotted into nitrocellulose membrane pore size 0.2 μ m (Schleicher & Schuell). After drying, we incubated the membrane overnight at 4 °C in TBS, pH

7.4 containing 6% nonfat milk (Sigma-Aldrich). The membrane was washed three times in TBST (TBS + 0.1% Tween 20) then incubated for 1 h at room temperature in TBS containing 1% nonfat milk and protein A purified polyclonal IgGs (IgGs) against the adhesion domain (AD1–3) of HxfB (it recognizes both HxfA and HxfB) with concentration ≈ 5 μ g/mL (we estimated an A_{280} of 1.4 = 1 mg of IgGs). After three washings in TBST, we incubated the membrane for 1 h at room temperature with alkaline phosphatase-conjugated goat anti-rabbit IgGs (Sigma-Aldrich). We washed the membrane three times with TBST and detected antigen-antibody complexes with an alkaline phosphatase color developer (NBT/BCIP) according to the manufacturer's instructions (Sigma-Aldrich).

Quantitative Real-Time RT-PCR. We obtained purified total RNA from samples by using TRIzol Plus RNA Purification Kit (Invitrogen). The c-DNA was synthesized using 5 μ g of total RNA with the SuperScript first-strand synthesis system (Invitrogen) with random hexamer primers as described by the manufacturer's instructions. We performed real-time PCRs in 20- μ L reactions comprising 5 pM each forward and reverse primers (4), cDNA template, and 2 \times SYBER Green PCR master mix (Applied Biosystems). We used the 16S rRNA gene as an endogenous control to normalize samples. PCR was performed on an ABI 7500 Fast Real-Time PCR System (Applied Biosystems) with cycling parameters as previously described (4). We performed each PCR in triplicate and the experiments were repeated three times. Data were analyzed by SDS relative quantification software (Applied Biosystems).

1. Almeida RPP, Purcell AH (2006) Patterns of *Xylella fastidiosa* colonization on the precarbarium of sharpshooter vectors relative to transmission to plants. *Ann Entomol Soc Am* 99:884–890.
2. Killiny N, Almeida RPP (2009) *Xylella fastidiosa* afimbrial adhesins mediate cell transmission to plants by leafhopper vectors. *Appl Environ Microbiol* 75:521–528.
3. Roper MC, Greve LC, Labavitch JA, Kirkpatrick BC (2007) Detection and visualization of an exopolysaccharide produced by *Xylella fastidiosa* in vitro and in planta. *Appl Environ Microbiol* 73:7252–7258.
4. Chatterjee S, Wistrom C, Lindow SE (2008) A cell-cell signaling sensor is required for virulence and insect transmission of *Xylella fastidiosa*. *Proc Natl Acad Sci USA* 105:2670–2675.

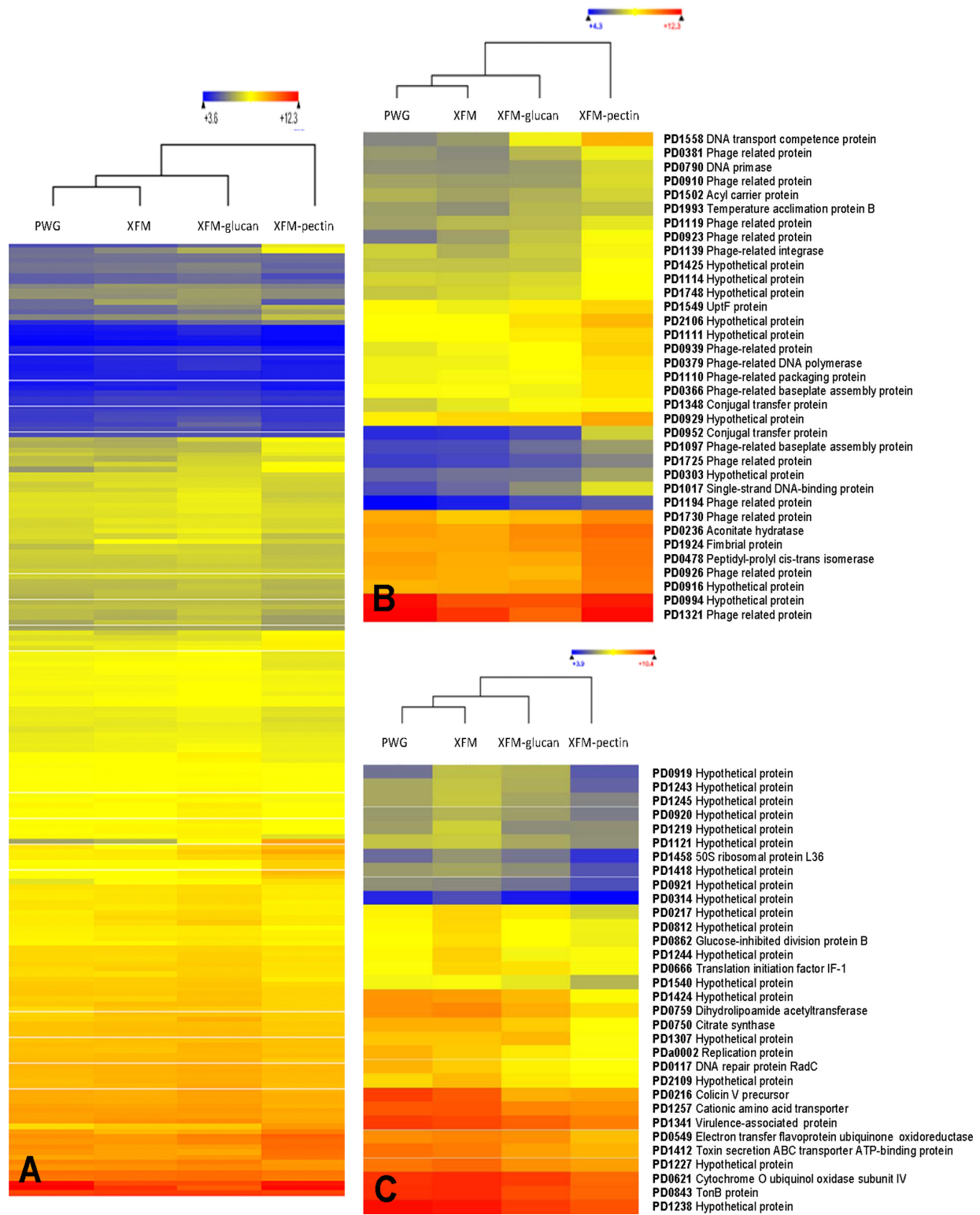


Fig. S1. Microarray analysis for gene expression of cells grown in different media. (A) Hierarchical clustering analysis of microarray expression data for 187 genes found to be differentially regulated during growth in PWG, XFM, XFM-pectin, and XFM-glucan, respectively. (B and C) Up-regulated genes in the presence of pectin (B) and down-regulated genes in the presence of pectin (C) compared with XFM. Note the increasing or decreasing gradient of expression data are the average of four independent replicates for each treatment. The most intense red and blue colors correspond to increased or decreased expression values respectively. Genes having ratios of ≥ 1.6 - or ≤ 0.6 -fold for expression were selected as up-regulated or down-regulated, respectively.

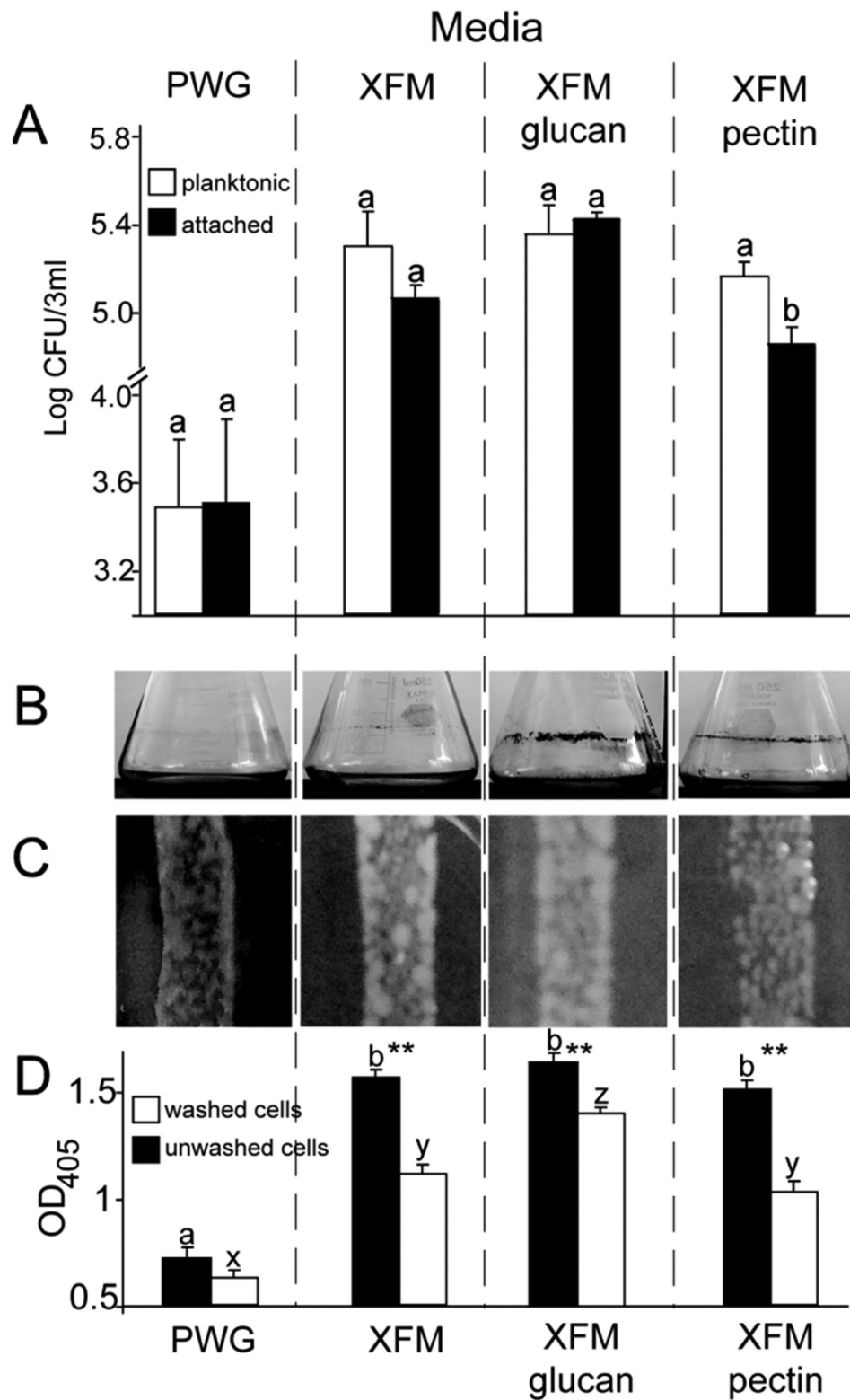


Fig. S2. Growth characterization of *X. fastidiosa pgIA* mutant grown in four different media. (A) Populations of planktonic versus glass-attached cells grown in vitro, bars with the same letter are not different from each other within media treatments (*t* test, $P < 0.05$). (B) Biofilm formation at air/broth interface in different media. (C) Visual aspect of bacterial lawns on solid media; glossy phenotype likely associated with EPS production. (D) EPS production quantified immunologically in four media (filled bars, unwashed cells; empty bars, washed cells). Asterisks ($P < 0.05$ for one, $P < 0.001$ for two) indicate within media differences; bars with the same letter are not different from each other within wash treatments.

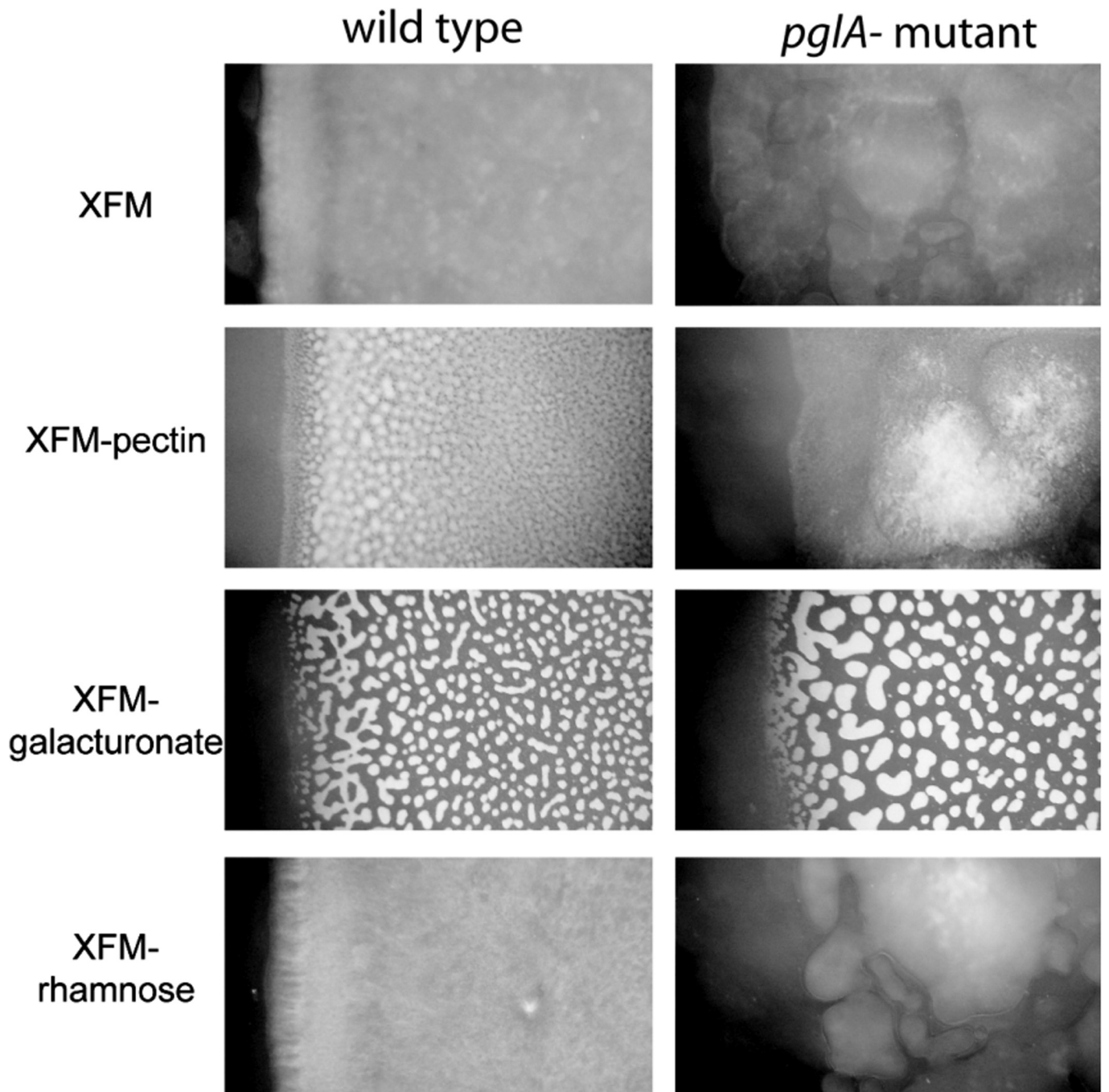


Fig. S3. Phenotype of wild-type and *pglA* mutant colonies on XFM media supplied with pectin or one of its major components. Pictures focus on the edge of plated cells for comparative purposes. Images were taken by using a LEIKA M125 stereomicroscope with 100× total magnification. Inocula were adjusted to OD₆₀₀ of 0.2 before plating on the media.



Fig. S4. Artificial diet system used to perform *X. fastidiosa* transmission assays.

Other Supporting Information Files

[Table S1 \(PDF\)](#)

[Table S2 \(PDF\)](#)

[Table S3 \(PDF\)](#)