

The following preliminary results are presented to support our FCPRAC preproposal on resistance to bacterial diseases of citrus.

Table 1. Sequence Relatedness Among Four Essential Genes of Four Bacterial Pathogens of Citrus and a Pathogen of Cabbage and Arabidopsis (Xcc).

Pathogen/ Disease	GeneA ¹	GeneB ¹	GeneC ¹	GeneD ¹
Xac ² /canker	100	100	100	100
Xcc/black rot	92 ³	99	92	93
CLa/greening	NA ⁴	78	NA	NA
Xf/CVC	75	95	79	75
Sc/stubborn	NA	72	NA	70

¹ The identity of genes A, B, C and D are being withheld pending patent filing.

² Abbreviated names for the bacterial plant pathogens (and disease caused)

Xac = *Xanthomonas axonopodis* pv. *citri* (citrus canker)

Xcc = *Xanthomonas campestris* pv *campestris* (cabbage black rot)

CLa = *Candidatus Liberibacter asiaticus* (citrus greening)

Xf = *Xylella fastidiosa* (citrus variegated chlorosis; CVC)

Sc = *Spiroplasma citri* (citrus stubborn)

³ Indicates the percentage identity of that pathogen's gene sequence to the homologous gene in the citrus canker pathogen, Xac.

⁴ This gene sequence is not currently available, but sequencing is ongoing, and the entire sequence of CLa will soon be available.

The very high levels of identity among the sequences of Xac and Xcc genes A, B, C and D give confidence that the information gained with Arabidopsis and Xcc experiments will result in resistance to Xac, and likely to the other three citrus bacterial pathogens as well.

Table 2. Viability of *Xanthomonas campestris* pv *campestris* (Xcc) cells following exposure to various RNAs. Cells were incubated *in vitro* in solutions of double-stranded RNAs (dsRNA) or small Interfering RNAs (siRNA) prepared from Xcc Gene A or Gene B.

A. Viability of Xcc cells as measured by colony formation following treatments¹

Treatment	# Colonies formed	% Reduction in colony formation
Control (no RNA)	111	0
Unrelated gene dsRNA ²	102	8
Unrelated gene siRNA ³	100	10
Gene A dsRNA ²	77	30
Gene A siRNA ³	56	50
Gene B dsRNA ²	84	24
Gene B siRNA ³	49	55

¹ Xcc cells (10^6) were incubated in solutions of the dsRNA or siRNA for 16 hours and plated on YDC medium. The average number of colonies for three replicates is given.

² Xcc cells were incubated in 35 ug of dsRNA in 30 ul

³ Xcc cells were incubated in 10 ug of siRNA in 30 ul

B. Viability of Xcc cells as measured metabolically following treatments⁴

Treatment	Differential absorbance	% Reduction in viability
Control (no RNA)	0.117	0
Gene A siRNA ⁵	0.076	35
Gene B siRNA ⁵	0.079	33

⁴ Cell viability was determined using the Promega CellTiter 96 assay where the reduction of a tetrazolium compound by living cells is measured directly by the differential absorbance at 490 and 650 nm

⁵ Xcc cells (10^5) were incubated in 10 ug of siRNA in 30 ul for 16 hours

These assays indicate that 24 to 55% of the Xcc cells are killed or inactivated by incubation in solutions of dsRNAs or siRNAs from Xcc gene A or gene B. The siRNAs are more active at lower concentrations. This indicates that genes A and B are excellent candidates to test for conferring resistance to Xcc, and then to the citrus bacterial pathogens.